THE EFFECTS OF HAIR CELL DAMAGE ON COCHLEAR FUNCTION

by

R. V. HARRISON

A dissertation presented to the University of Keele for the degree of Doctor of Philosophy.

March, 1978.

Department of Communication
and Neuroscience
University of Keele.

ORIGINAL COPY IS TIGHTLY BOUND AND TEXT IS CLOSE TO THE EDGE OF THE PAGE

ABSTRACT

The main aim of this study was to investigate the threshold and tuning properties of cochlear nerve fibres in kanamycin treated guinea pigs having various degrees of hair cell loss restricted to outer hair cells (OHCs).

The frequency threshold curves (FTCs) of fibres originating in regions of total OHC loss had thresholds elevated 40-50 dB above normal, and a broadening of their 10 dB bandwidths, on average, to five times greater than normal. On the assumption that the inner hair cells (IHCs) in the regions of OHC loss are unaffected by the kanamycin, the results suggest that the normal low threshold, sharply tuned properties of cochlear fibres depend in some way on the integrity of the OHCs. Because the majority, if not all, of these fibres originate at the IHCs, some form of interaction between outer and inner hair cells and /or their innervations is implicated.

Also investigated was the relationship between the elevation of minimum thresholds of FTCs and their 10 dB bandwidths. For fibres with characteristic frequencies (CFs) above 2 kHz, the relationship was non-linear such that the 10 dB bandwidth was substantially increased only for threshold elevation greater than 30-40 dB. For lower CFs, 10 dB bandwidths increased hand in hand with minimum theshold elevation.

The physiological findings are discussed in relation to possible mechanisms of sharp cochlear tuning, and also to possible correlates in psychophysical studies of deafness of cochlear origin.

Cochlear action potentials (CAPS) were also recorded from normal and kanamycin treated GPs. Latency and amplitude: intensity functions were measured (to empirically test theories of how they reflect underlying cochlear fibre activity) and also CAP thresholds to tone pip stimuli. The latter thresholds correlated well with the minimum thresholds of corresponding cochlear fibres over a frequency range of 1-40 kHz, for both normal and pathological cochleas.

ACKNOWLEDGEMENTS.

The experiments described in this dissertation were carried out in the Research Department of Communication and Neuroscience, and I am indebted to the head of that Department, Professor D.M. Mackay, and to my research supervisor, Dr. E.F. Evans, for the facilities they provided. The support of the Science Research Council, through a research studentship, is gratefully acknowledged. I also acknowledge a grant from the Medical Research Council to Dr. E.F. Evans which provided some of the equipment used in this study.

My very special thanks go to Dr. Evans for his invaluable guidance and advice on this project, and for the excellent scientific example he has set.

I would like to thank other members of the Department, in particular Dr. Graham Pick and Alan Palmer, for their technical advice, and for the many hours spent with them in useful discussion. I am also grateful to Mrs. Janet Bailey for her enthusiastic assistance in rearing and maintaining the guinea pigs used in this study.

Finally, my sincere thanks go to Judith, my wife, both for typing this dissertation and for her unfailing support and encouragement.

CONTENTS

CHAPTER 1.

INTRODUCTION.

Within the ears of men, and without their knowledge or contrivance, this lute of 3,000 strings has existed for ages, accepting the music of the outer world and rendering it fit for reception by the brain ..... surely, inability to feel the stupendous wonder of what is here revealed would imply incompleteness of mind.

JOHN TYNDALL : ON SOUND. 1893. A reference to the organ of Corti.

1.1a GENERAL INTRODUCTION TO THE MAIN AIMS OF THE PRESENT STUDY.

This introductory section briefly presents the background to the main questions addressed in the present study and also outlines the strategy taken to answer these questions. The more important aspects are considered in more detail in the other sections of this first chapter.

The sensory hair cell is undoubtedly the most important component of the acoustico - lateralis system in vertebrate animals, being ultimately responsible for the excitation of sensory neurones in response to the energy of mechanical vibration. The mammalian cochlea is characterized by its possession of two distinct, separately innervated, hair cell populations, the inner hair cells (IHCs) and the outer hair cells (OHCs), and the prime aim of this study was to investigate the rôle of the OHCs in normal cochlear function. Of particular interest is the contribution of the OHCs to the threshold and tuning properties of the cochlea, properties that are manifest in the low threshold and sharply tuned frequency threshold curves[1] (FTCs) recorded from single cochlear fibres (e.g. in cat: KATSUKI et al. 1958; KIANG et al. 1965; and guinea pigs (GP): EVANS, 1970, 1972). The upper curves in figure 1.1 (from EVANS, 1972) are eight typical FTCs from normal GPs, and they illustrate how each fibre is tuned to a narrow range of stimulus frequencies.

Because 85-95% of afferent cochlear fibres appear to terminate on the IHCs (SPOENDLIN, 1970, see figure 1.4; MORRISON et al. 1975) it is likely that the great majority of the recordings from cochlear fibres have been made from fibres originating on the IHCs. Indeed the absence of any anatomical evidence for more than one population of afferent fibres based on fibre diameters (GACEK & RASMUSSEN, 1961; HALL & RØNNING-ARNESEN, 1974) and the lack of clear physiological evidence of more than one population (the responses of cochlear

---

1 The frequency threshold (tuning) curve of a cochlear fibre is the threshold boundary of its response area in frequency and intensity space. Stimuli with frequency and intensity combinations falling within the FTC cause an increase in the fibre discharge rate above its spontaneous rate of activity.

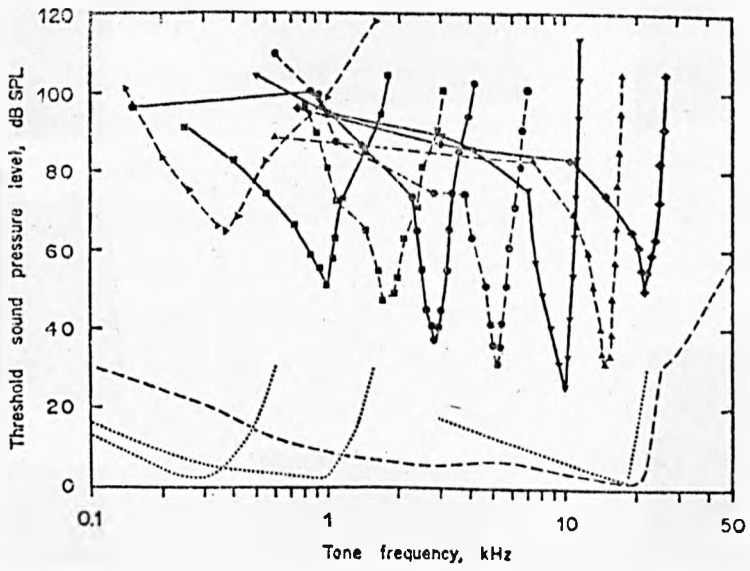


FIGURE 1.1 (from EVANS, 1975c). Frequency threshold curves of 8 cochlear fibres from 6 guinea pigs, in dB SPL measured at the tympanic membrane (corrected to closed bulla conditions). Curves below: analogous curves derived from the measurements of the vibration amplitude of the guinea pig basilar membrane by VON BÉKÉSY (1944, curves at 0.27 and 0.9 kHz), by JOHNSTONE et al. (1970, curve at 18 kHz) and by WILSON & JOHNSTONE (1972, dashed curve at 20 kHz). These curves are corrected for the GP middle ear frequency response to relate them to the sound pressure at the tympanic membrane and are positioned arbitrarily on the intensity scale.

fibres are relatively homogeneous e.g KIANG, 1968; EVANS, 1972; see section 1.32) suggest that all the fibres recorded from, may originate at the IHCs. However, from a number of considerations, the OHCs have been held responsible for the low thresholds of cochlear fibre responses and for their sharp tuning. The evidence in favour of this hypothesis (as well as contrary evidence) is given in section 1.3d.

Of particular importance to the question of the contribution of the OHCs to normal cochlear fibre responses, are studies which have revealed the vulnerability of the low threshold and sharp filtering properties of cochlear fibres to various kinds of acute cochlear pathology, e.g. cochlear hypoxia (EVANS, 1970, 1972, 1974a, 1974b; ROBERTSON & MANLEY, 1974), cyanide and furosemide poisoning (EVANS & KLINKE, 1974; EVANS, 1974a, 1974b, 1975b,c). One result of such cochlear insults was a loss of the low threshold and sharply tuned segment of the cochlear fibre FTC, leaving behind a high threshold, less well tuned FTC. On the grounds that the OHCs may be more vulnerable than the IHCs to such cochlear insults (and on the other grounds reviewed in section 1.3d) the speculation was made that the low threshold and sharp tuning properties of cochlear fibres were in some way dependent upon the integrity of the OHCs. The speculation found some support in the results of a study by KIANG, MOXON & LEVINE (1970) which indicated a deterioration in the threshold and tuning properties in cat cochlear fibres from cochlear regions with severe OHC loss caused by kanamycin poisoning, but where IHCs remained apparently intact. However, KIANG et al. did not draw this conclusion themselves, and have expressed reservations (KIANG et al. 1976) on the grounds that the extent of the region over which total OHC loss occurred was small, and a precise cochlear frequency map for the cat was not available. This made it difficult to correlate, with certainty, changes in the FTCs with the region of OHC loss.

The present study therefore sought to examine, under more favourable conditions, the effects of hair cell loss, restricted to OHC, on the threshold and tuning properties of cochlear fibres. These more favourable conditions were produced by using the GP as the experimental animal. It was chosen because:

a) A directly measured cochlear frequency map existed in our laboratory for the GP (WILSON & JOHNSTONE, 1972; methods section 2.9).

b) Large areas of hair cell loss, restricted to OHCs, can be induced in the GP with chronic administration of kanamycin (e.g HAWKINS & ENGSTRÖM, 1964; KOHONEN, 1965; DALLOS & WANG, 1974; YLIKOSKI, 1974; section 1.4).

c) Surface preparation techniques are particularly easy to apply in the GP (ENGSTRÖM et al. 1966; methods section 2.8). By this method, the pattern of hair cell degeneration caused by kanamycin poisoning was most easily assessed.

The main problem with selecting the GP was anaesthesia. Under conventional (e.g. barbiturate) anaesthesia, cardiovascular and respiratory depression is extremely common, and can lead to a deterioration in cochlear threshold sensitivity and tuning properties (see EVANS, 1972). To overcome these problems a new anaesthetic technique was developed, utilizing neuroleptanaesthesia. (The method was suggested by, and developed in collaboration with E.F. EVANS. It is fully described in methods section 2.3). This anaesthetic technique, together with strict control of blood pressure and respiration, and careful monitoring of cochlear condition (see methods) has enabled the present experiments to be carried out without the problems caused by acute cochlear hypoxia, the outcome of which would make the interpretation of the results from long term pathology impossible.

To test the hypothesis that the OHCs are responsible for normal thresholds and tuning properties of cochlear fibres, these properties have been measured in cochlear fibres which originate in cochlear regions with total OHC loss caused by kanamycin poisoning. The responses of fibres from cochlear areas with various degrees of OHC loss were also investigated. In all these circumstances, the crucial assumption is being made that the IHCs in such areas (which appear normal under light microscopy) are not themselves damaged by kanamycin. This assumption is critically examined in section 1.4d. In brief, much evidence in support of this assumption comes from electronmicroscopic (EM) studies of the hair cells which remain after kanamycin poisoning. Thus LUNDQUIST & WERSÄLL (1966) found few abnormalities in such cells. YLIKOSKI (1974) reported that providing about four weeks are allowed as a recovery period after kanamycin administration, there were no gross morphological changes in IHCs remaining in regions of total OHC loss.

The main aim of the present study was to investigate the mechanism of sharp tuning in normal, low threshold cochlear fibres by which means the cochlea fulfils its rôle as a frequency analyzer. As a background against which the results of the present study will be discussed (chapter 7.0) it is appropriate to present a brief review of the prevailing ideas concerned with cochlear frequency analysis, and also a brief summary of the features of cochlear anatomy and innervation which may be relevant to this mechanism. These reviews are given in sections 1.2 and 1.3 of this introduction.

1.1b SECONDARY AIMS OF THE PRESENT STUDY.

The secondary aim of the present study was to investigate how cochlear action potential (CAP) responses were related to single cochlear fibre recordings in the same animal, and also to the state of cochlear hair cell degeneration. (The various patterns of hair cell degeneration produced in the GPs of this study were well defined by the histological evaluation

methods.) Two aspects of CAPs are investigated:

a) The thresholds of CAPs to frequency specific (tone pip) stimuli. The question asked was: how far can CAP thresholds serve as an indication of individual cochlear fibre thresholds in the normal cochlea and under various conditions of hair cell loss? The reasons for this investigation were two fold. Firstly, in clinical electrocochleography, tone pip stimuli have proved useful for determining thresholds of cochlear response across frequency (DAVIS, 1976; EGGERMONT & ODENTHAL, 1974; SCHMIDT, EGGERMONT & ODENTHAL, 1974). There has however, been no direct evidence that tone pip evoked CAPs can be valid indicators of the thresholds of individual cochlear fibres, and hence, in cochlear pathology, residual cochlear function. Direct evidence, albeit in GP, is being sought in this study. Secondly, in experimental investigations on cochlear function, the physiological condition of the cochlea is a potential variable and should be rigorously controlled. One of the earliest manifestations of a physiological deterioration is an elevation of the minimum threshold of cochlear fibre responses (e.g. EVANS 1970, 1972). In this respect, the ability to determine such threshold elevation as a function of frequency using grossly recorded CAP thresholds to tone pips is very useful, particularly in experiments, such as the main part of the present study, where confusion could arise between threshold elevation caused by chronic cochlear pathology and accidental acute cochlear deterioration during experimental proceedings. For such use, the relationship between cochlear fibre minimum thresholds and the frequency specific CAP thresholds needs to be demonstrated.

b) The amplitude:intensity and latency : intensity functions of the N_1 (first negative going peak) of the CAP in response to click and tone pip stimuli.

These functions are commonly measured in human electrocochleography for diagnostic purposes (e.g. YOSHIE, 1968; ARAN, 1971, 1973; SALOMON & ELBERLING, 1971; EGGERMONT & ODENTHAL, 1974; MONTANDON et al. 1975a, b,). However, their proper interpretation is prevented because it is still unclear as to how the functions (particularly those from pathological cochleas) reflect the underlying activity of cochlear fibres, and in the case of cochlear pathology, the extent and degree of the cochlear lesion.

A number of suggestions and theories have been proposed to explain the forms of the amplitude and latency : intensity functions. These range from those based on the improbable assumption of two populations of cochlear fibres (e.g DAVIS et al. 1958; YOSHIE, 1968; ARAN, 1971; see section 8.2 for other references), to more likely suggestions which involve known properties of cochlear fibre responses[2] (EVANS, 1975b; ÖZDAMAR & DALLOS, 1976; De BOER, 1975,

---

2 Rather than describe the suggestions of these authors in this introduction, it is more appropriate and convenient to do so in discussion (section 8.2).

1977). De BOER'S model has incorporated a number of the properties of cochlear fibres (e.g their frequency selectivity and latency of response) and can reproduce some (but by no means all) features of the normal latency and amplitude : intensity functions. The present investigation was an attempt to test empirically these theories, particularly their prediction of the shape of functions in cochlear pathology involving OHC loss.

By comparing amplitude : intensity functions (for tone pip and click stimuli)with the extent of hair cell damage in the cochlea, it was also possible to ascertain which features of such functions were reliable, and therefore useful indicators of the degree or pattern of a cochlear lesion.

The results and discussion of this study on cochlear action potentials are presented in chapters 5 & 8 respectively.

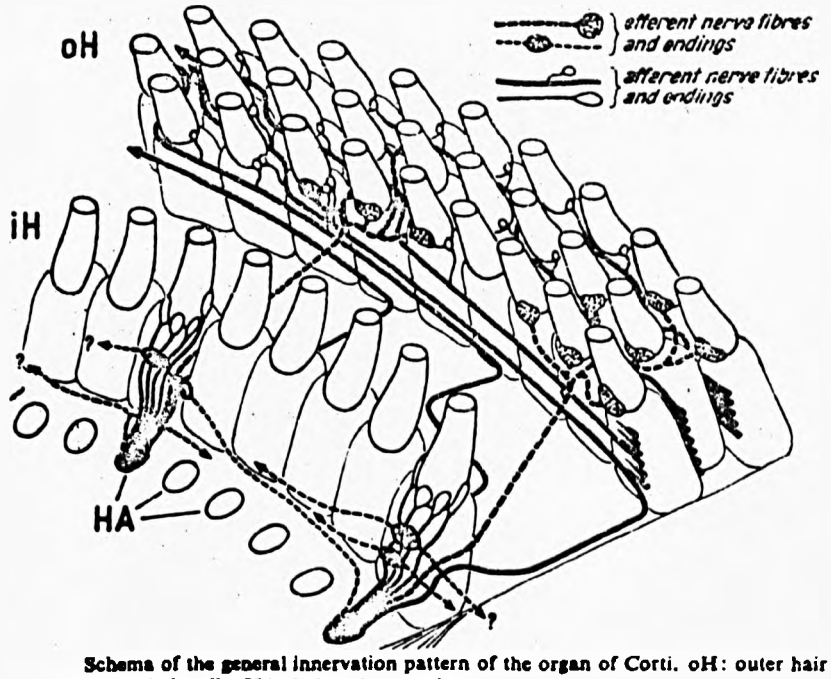
An essential part of any study concerned with the investigation of abnormal function is to define what is normal by the use of control studies carried out under the same conditions. Thus, data from normal control animals are presented, mainly for comparison with results from pathological animals. Certain aspects of the normal data are, however, discussed in their own right, particularly where they differ, or where the conclusions based on them differ, from the findings of others.

1.2. COCHLEAR INNERVATION.

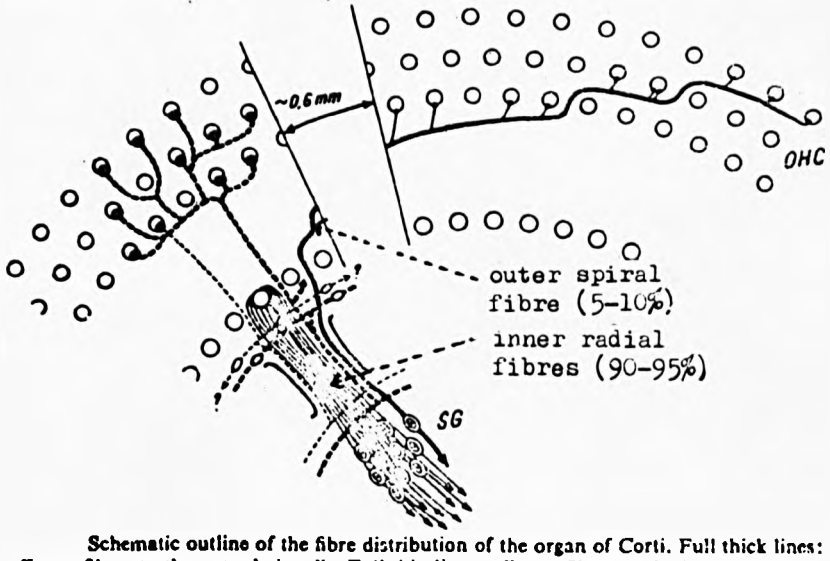
The present study is primarily concerned with a correlation of cochlear nerve fibre responses and the pattern of hair cell damage in the cochlea. It is appropriate therefore, to briefly summarize what is presently known about the afferent innervation of the cochlea.

Figure 1.2 (from SPOENDLIN, 1970) summarizes the scheme of innervation for the cat cochlea. There are two main types of afferent fibre, the inner radial fibres which innervate only the IHCs, and which make up the majority of the afferent fibres, and the outer spiral fibres which innervate the OHCs. In the cat, 90-95% of afferents are inner radial fibres, and only 5-10% are outer spiral fibres (SPOENDLIN, 1970). In the guinea pig, according to MORRISON et al. (1975), 85-90% of the afferents are inner radial fibres and 10-15% are outer spiral fibres.

Both types of afferent fibres enter the organ of Corti via the habenula perforata, and so through each habenular opening pass some 20 inner radial fibres along with only one or perhaps two fibres destined for the OHCs (SPOENDLIN, 1970). All the fibres are unmyelinated as they enter the habenula perforata amd remain so in the organ of Corti. As shown in figure 1.2 , the inner radial fibres terminate immediately on the IHCs, whilst the outer spiral



Schema of the general innervation pattern of the organ of Corti. oH: outer hair cells; iH: inner hair cells; HA: habenular openings.



Schematic outline of the fibre distribution of the organ of Corti. Full thick lines: afferent fibres to the outer hair cells. Full thin lines: afferent fibres to the inner hair cells. Interrupted thick lines: efferent nerve fibres from the contralateral olivo-cochlear bundle Thin interrupted lines: efferent nerve fibres from the homolateral olivo-cochlear bundle.

FIGURE 1.2 (from SPOENDLIN, 1970). Schematic diagrams of the innervation pattern of the (cat) organ of Corti. Dashed lines represent efferent fibres, solid lines represent afferent fibres. Of the afferent fibres, 90-95% are inner radial fibres innervating the IHCs, and only 5-10% are outer spiral fibres innervating the OHCs.

fibres cross the tunnel of Corti and then take a spiral course basalward. The outer spiral fibres are firmly embedded in the cytoplasm of the tunnel floor and are sometimes termed basilar fibres at this point. When they reach the OHC region, each outer spiral fibre typically runs basalward for up to 0.7 mm (as inferred from electronmicrographic (EM) sections by SPOENDLIN 1970) before terminating on about 10 OHCs. PERKINS & MOREST (1975) used Golgi staining (of the kitten cochlea) to visualize individual outer spiral fibres. They drew attention to the great variation in the length of the outer spiral fibres and the number of the OHCs innervated. They found outer spiral fibres of up to 0.5 mm in length in the kitten (up to 0.7 mm in the rat). They also noted that for fibres which innervated only one row of OHCs, this was usually row 1 of the OHCs, and that fibres terminating on 2 rows of hair cells usually did so on rows 2 & 3 of the OHCs. (table 4 in PERKINS & MOREST, 1975).

In the GP, SMITH (1975) used the same staining methods, and also found a considerable variation in the length of the outer spiral fibres (0.2 - 0.7mm). Furthermore, she reported a systematic variation from apex to base in the extent of outer spiral fibre innervation, the apical outer spiral fibres more frequently innervating a longer segment of the organ of Corti than in the basal turn. In the base they branched over approximately 0.1 mm and innervated 6-20 hair cells consecutively, whereas apically the same number of hair cells were innervated but usually non-consecutively, and therefore over a longer segment of the organ of Corti. SMITH also found that the outer spiral fibres in the basal turn usually innervated a single row of OHCs whereas more apically there was a tendency for all three rows to be innervated by a single fibre.

All these afferent fibres have their cell bodies in the spiral ganglion, located in Rosenthal's canal. The axons of these spiral ganglion cells congregate in the modiolus, and run together (with a spiral, tonotopic organization) through the internal auditory meatus to the cochlear nucleus. There is no histological evidence to suggest that, from their origin at the hair cell(s) to their termination at the cochlear nucleus, the afferent neurones synapse with any other type of neurone or with each other.[3] In particular, there is no evidence of synaptic regions at the habenula perforata where unmyelinated outer spiral fibres and inner radial fibres run closely together (e.g SPOENDLIN, 1966, 1970, 1973, 1974). Any direct interaction between these fibres could, if at all, only occur on an electrical basis (SPOENDLIN 1970).

---

3 The only morphological evidence which could possibly indicate a neural interaction has come from PERKINS & MOREST (1975) who found that some outer spiral fibres send branches to IHCs via short collaterals. However, these have only been found in the cochleas of immature cats and rats. SMITH (1975) using the same GOLGI staining method in adult GP, and SPOENDLIN e.g. (1973) in the cat have failed to find similarly branched outer spiral fibres.

1.3a COCHLEAR FREQUENCY ANALYSIS

The validity of the 'place theory'[4] of cochlear frequency analysis is now well established, particularly by the work of VON BÉKÉSY who demonstrated that the maximum of the travelling wave displacement envelope of the basilar membrane (BM) shifts with frequency of stimulation. High frequencies and low frequencies cause maximal disturbance in basal and apical regions of the basilar membrane respectively. In direct observation of the movement of the cochlear partition (under stroboscopic illumination) VON BÉKÉSY was able to measure the basilar membrane amplitude vibrations for low frequencies and to demonstrate its mechanical tuning properties (review: BÉKÉSY, 1960). These basilar membrane measurements in guinea pig were extended to higher frequencies by JOHNSTONE et al. (1970) using a Mössbauer technique, by WILSON & JOHNSTONE (1972, 1975), with a capacitance probe, and by KOHLLÖFFEL(1972) using laser fuzziness detection. The measurements of mechanical tuning in the guinea pig cochlea from the above methods are in good agreement and also compare well with the data of DALLOS (1973) in which the mechanical frequency response of the GP cochlea was inferred from differential electrode recordings of cochlear microphonic (CM). Similarly, KOHLLÖFFEL (1971) found that the CM distribution pattern along an array of twelve electrodes was consistent with BM displacement (see WILSON, 1974 for discussion)

The frequency threshold curves (FTCs) or tuning curves of individual fibres in the cochlear nerve, represent the frequency selectivity of the cochlea, i.e. its ability to separate out frequency components in a complex signal. The first recordings from single auditory nerve fibres by TASAKI (1954) in the GP indicated the FTCs to be as broadly tuned as the mechanical BM tuning data of BÉKÉSY. However, subsequent single cochlear fibre recordings in cat and monkey by KATSUKI et al. (1958, 1961) and in cat by KIANG and his co-workers (KIANG et al. 1965, 1967) revealed more sharply tuned FTCs. Single cochlear fibre recordings in GP (EVANS 1970, 1972) extended the observation of sharp neural tuning[5] to the species on which most basilar membrane tuning data had been obtained. Figure 1.1 (from EVANS 1975b) allows a comparison between the FTCs from normal single cochlear fibres with analogous BM amplitude plots, and clearly shows the difference between mechanical tuning

---

[4] In 'place theory', the frequency of a sound stimulus is coded in terms of the position along the cochlear partition which that stimulus maximally excites. At low frequencies of stimulation, the place coding is possibly supplemented by a temporal analysis of the stimulus wave - form (as in the 'volley theory' of WEVER & BRAY, 1930).

[5] The reason for TASAKI's broadly tuned FTC is unclear. However, the most likely possibilities are that either acute cochlear hypoxia occurred during the experiment, and the FTC was therefore of raised minimum threshold and broadly tuned, or that the FTC represented an incomplete analysis, perhaps because contact with the fibre was lost. (EVANS, 1972).

data and neural tuning. The difference has been demonstrated conclusively in the cat by the concurrent measurement of BM tuning and cochlear fibre tuning (EVANS & WILSON, 1973).

1.3b. EVIDENCE FOR A SECOND FILTER.

The difference between the measured mechanical tuning of the BM and the neural tuning supports the hypothesis of a two stage filtering process in the cochlea, the first stage provided by the mechanical tuning of the BM, and the second to account for the sharply tuned responses of cochlear fibres (EVANS, 1972; WILSON & JOHNSTONE, 1972; EVANS & WILSON, 1973; review: EVANS 1975a).

The earliest[6] experimental evidence in favour of this hypothesis came from studies on two phenomena which are related to cochlear non-linearities i.e. the cubic difference tone $2f_1 - f_2$, and two tone suppression. Psychophysical investigations of $2f_1 - f_2$ (GOLDSTEIN, 1967, 1970; SMOORENBURG, 1972a, b) and investigations of its neurophysiological correlate (GOLDSTEIN & KIANG, 1968; GOLDSTEIN 1970) have demonstrated it to be a distortion product whose level is relatively independent of the level of the primaries, f_1 and f_2, which produce it (an 'essential' non-linearity) but dependent on the frequency separation of those primaries. The latter property indicates that the non-linearity is preceded by a frequency selective process. The $2f_1 - f_2$ behaves as if produced by a stimulus tone with a frequency of $2f_1 - f_2$, that is, it seems to be transduced in the cochlea at the position where an equivalent real tone would be. However, there is no evidence of a corresponding $2f_1 - f_2$ component in direct BM amplitude measurements[7] (WILSON & JOHNSTONE, 1972, 1973; RHODE, 1977) or in the BM mechanics inferred from CM recordings (DALLOS, 1969, 1973; DALLOS & CHEATHAM, 1974). Thus the non-linearity producing the $2f_1 - f_2$ cubic difference tone would appear to arise after the BM tuning.

---

6 Cochlear mechanisms which involve a sharp filtering mechanism in addition to the mechanical tuning of the BM had been previously proposed (e.g. GOLD, 1948; HUGGINS & LICKLIDER, 1951, FURMAN & FRISHKOPF, 1964), but were theoretical rather than based on experimental evidence.

7 GREENWOOD (1977 comment on RHODE 1977) has claimed that from estimates of the level of the $2f_1 - f_2$ cubic difference tone (from psychophysical and physiological studies) the negative results from direct BM measurements would be expected i.e. the $2f_1 - f_2$ is too far below the primaries to be detected (50-70 dB at high primary levels). However WILSON (1977 - discussion on RHODE 1977) maintained that with capacitive probe measurements, the $2f_1 - f_2$ was not found even at levels 10-20 dB below those expected on the basis of their neural thresholds in the same species.

However, in order to account for the frequency analysis of the cubic difference tone, to separate it out from its primaries, the non-linearity must be followed by a second frequency selective process (SMOORENBURG, 1972b).

Two tone suppression, also related to a cochlear non-linearity has also been accounted for in a model which involves enclosing a non linear section between two linear filters (PFEIFFER, 1970).

A number of other observations also support the two stage filtering hypothesis. The hypothesis accounts for the fact that each afferent fibre has its own 'private' filtering and threshold characteristics (EVANS, 1972; EVANS & WILSON, 1973) i.e fibres (in single animals) with similar characteristic frequencies (CFs) have bandwidth and cut-off slope values which vary widely. Thresholds also vary by as much as 20-30 dB in adjacent fibres, and occasionally (at least in the GP) fibres with greatly elevated thresholds (e.g. minimum thresholds greater than 70 dB SPL) have been found amongst a population of otherwise normal fibres (EVANS 1972). It is very unlikely that the variation in responses observed could be accounted for by the mechanical filtering properties of the BM which one would expect to be more evenly distributed.

Further evidence to suggest that the neural tuning has a separate mechanism from that of the BM tuning is the demonstration of the vulnerability of sharp neural tuning to physiological insults such as brief periods of hypoxia (EVANS, 1974a; ROBERTSON & MANLEY, 1974) and also acute intra-cochlear and systemic administration of ototoxic drugs (EVANS, 1974b, 1975bc, 1976a; EVANS & KLINKE, 1974). Figure (1.3) shows a result from the study by EVANS (1974a). Note how the sharply tuned segment of the FTC is lost (over a period of about 4 minutes) during hypoxic conditions (curves A to E) and subsequently recovers when normal oxygenation of the blood occurs (curves E to F). The BM tuning is unlikely, in such a short time scale, to be susceptible to such physiological insult.

RHODE (1973), investigating BM mechanics in the squirrel monkey with the Mössbauer effect, reported that small changes in the BM mechanics occurred within minutes of the death of the animal or removal of the cochlear nerve and cochlear blood supply. These changes were such as to reduce a non-linearity[8] of the BM mechanics which was reported to occur at moderate sound levels (c. 70 dB SPL) in the squirrel monkey, and which was claimed to be responsible for some degree of sharp mechanical tuning. However, these effects are too small in magnitude and not rapid enough to account for

---

[8] The non-linearity reported by RHODE (1971, 1973) in the squirrel monkey BM, and found at moderate sound pressure levels of 70 dB has not been found using capacitive probe methods in the GP at levels down to 40 dB SPL (WILSON & JOHNSTONE, 1972, 1975) or in the cat at levels of 55 dB SPL (EVANS & WILSON, 1975).

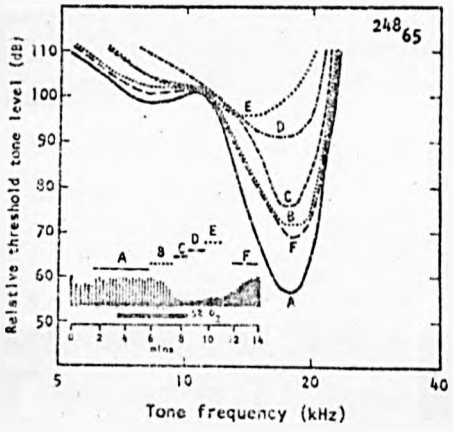


FIGURE 1.3 (from EVANS, 1974b). The reversible effects of hypoxia on a frequency threshold curve in the cat. The inset shows the times at which the curves were determined (in relation to the change in the cochlear action potential) before, during, and after a period of hypoxia produced by respiring the animal with an O_2 / N_2O mixture containing only 5% oxygen.

the neural tuning changes described above. On the other hand, WILSON & JOHNSTONE (1975) found that the BM mechanics, as measured with a capacitive probe, did not significantly alter after death or as a result of physiological changes which caused threshold shifts in CAP responses. Long term post-mortem changes (over days) which have been reported (e.g. KOHLLOFFEL, 1973; RHODE, 1973) are, of course, too slow to account for the relatively rapid neural tuning curve changes.

Figure 1.4 (from EVANS & WILSON, 1973) indicates, in summary, the possible arrangement, characteristics and location of the functional mechanisms required for cochlear frequency analysis.

1.3c POSSIBLE MECHANISMS FOR A SECOND FILTER.

A number of ideas have been put forward concerning the site and mechanism of the second filter. Some of these models propose that the ultimate mechanical stimulus to the hair cells is not represented by the travelling wave envelope but some derivative of it which is more frequency specific. Indeed, all the determinations of the amplitude of vibration of the BM have been made from the scala tympani and it is possible that the interaction of the BM motion with the tectorial membrane can produce more specific stimulation of hair cell than the whole travelling wave envelope.

Some models invoke purely mechanical processes such as the 'beam' or relative deformation hypothesis of HUGGINS & LICKLIDER (1951) whereby a relatively stiff tectorial membrane pulls at the stereocilia when the organ of Corti and the tectorial membrane move apart. By postulating that the mode of excitation is force rather than pure displacement, it is possible that sharpening can occur. Other purely mechanical models depend on the observations that various modes of shear occur at restricted points along the travelling wave. BÉKÉSY (1953) claimed that longitudinal and radial shear components occurred in the BM displacements, and analogues have also been demonstrated in cochlear models (TONNDORF, 1962; KHANNA et al. 1968). TONNDORF (1973, 1974) has proposed, on the assumption that hair cells respond optimally to shearing in one particular direction, that the organ of Corti could be stimulated over a more restricted distance, and provide neural tuning curves with steeper cut-off slopes than the amplitude tuning of the BM. However, as EVANS has pointed out (discussion on EVANS & WILSON 1973; discussion on TONNDORF 1974) this shearing mechanism cannot fully account for the steep low frequency cut-off slopes found in cochlear fibre FTCs of up to 200 dB/octave. Indeed the shear force envelopes demonstrated (by TONNDORF 1974) were not much different from the corresponding BM volume displacement envelopes. Similarly, mathematical models by BILLONE & RAYNOR (1973) based on anatomical considerations also provided estimations of shear force envelopes which were similar to corresponding BM volume displacement envelopes.

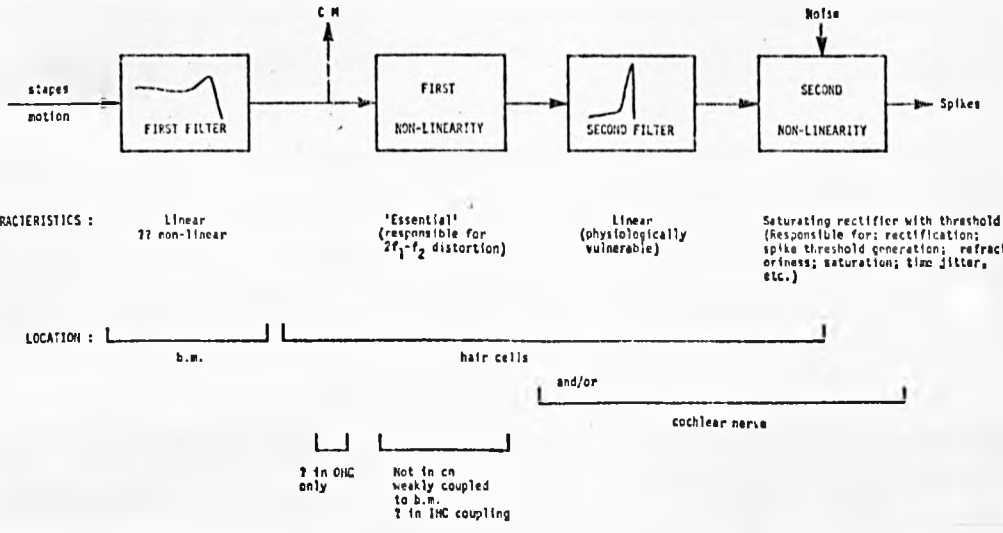


FIGURE 1.4 (from EVANS & WILSON, 1973). Summary scheme for the possible arrangement, characteristics, and location of the functional mechanisms of the cochlea.

Different hypotheses, based on observation of fluid streaming between the tectorial membrane and the reticular lamina, have been proposed (STEELE, 1973; HELLE, 1974; ZWICKER, 1974). These authors have observed, in cochlear models, localized changes in the gap dimensions, (in particular a gap closure) between the tectorial membrane and the reticular lamina which could give rise to localized fluid streaming across the reticular lamina. Clearly the free standing stereocilia of the IHCs (HOSHINO, 1977; LIM, 1977) would be most likely to be stimulated by such a fluid flow, although the short stereocilia of the OHCs which are not embedded into the tectorial membrane (KIMURA, 1966) could perhaps be displaced depending on the elasticity of the fibrils which connect the OHC stereocilia together (FLOCK, 1977). Even without considering fluid flow across the reticular lamina, the observed gap closure could be important as a mechanism to stimulate hair cells directly over a more restricted cochlear region than the overall displacement of the BM. Thus WILSON (comment on ZWICKER, 1974) speculated that perhaps the closure of the gap could cause a direct coupling of the tectorial membrane with the stereocilia of the IHCs, and if this coincided with a maximum shear motion it could provide maximal hair cell excitation compared with hair cells in neighbouring positions.

There are also theories which involve some filtering at the hair cell transduction stage. Hair cells have a directional sensitivity (LOWENSTEIN & WERSÄLL, 1959; BÉKÉSY, 1960; FLOCK, 1965) with the hair cell responding maximally to stereocilia displacement in the direction of the basal body. The effectiveness of stimulation decreases with a change in the direction of the displacement of the stereocilia, approximately as a cosine function (FLOCK, 1971). DUIFHUIS (1974) has proposed that a combination of this directional sensitivity with a directional distribution of vibration as a function of place (such as radial shear, described above e.g BÉKÉSY, 1953) could produce a filter. DUIFHUIS suggested that this could be an adequate second filter.

Unfortunately, it is almost impossible to test whether the above cited models involving mechanical filtering actually occur in the cochlea, because direct measurement of vibration in the region of the tectorial membrane is difficult. Furthermore, the essential stimulus could be very small compared with the overall amplitude of vibration of the BM which we know to be broadly tuned.

Some theories of the cochlear filtering mechanism subsequent to broad BM tuning involve a neural mechanism. Lateral inhibition has been suggested (BÉKÉSY, 1960; FURMAN & FRISHKOPF, 1964), however there is no evidence for a suitable innervation pattern and there is physiological evidence against such a mechanism (EVANS &WILSON, 1973). Other theories of filtering involving

neural organisation attempt to incorporate the known innervation patterns of the inner and outer hair cells. These will be considered in section 1.3e after reviewing the available evidence indicating the respective rôles of the inner and outer hair cell populations in normal cochlear function.

1.3d. THE RÔLES OF THE INNER AND OUTER HAIR CELL POPULATIONS.

Because the majority of cochlear afferent fibres innervate the IHCs, the majority of cochlear nerve fibre recordings (e.g.FTCs such as shown in fig. 1.1) are likely to have been made from fibres which originated from the IHCs. Indeed because there is no histological evidence for two populations of fibres in the auditory nerve (based on axon size) from either light microscopic (GACEK & RASMUSSEN 1961) or EM examination (HALL & RØNNING-ARNESEN 1974), it is possible that only axons of inner radial fibres are present in the nerve and that all recordings have been from these. Although rates of spontaneous activity and (within limits) the minimum thresholds and tuning of cochlear fibres vary[9] from fibre to fibre in the same animal, at similar CFs, there is little direct evidence to indicate two separate populations (e.g.KIANG et al. 1965; KIANG, 1968; EVANS, 1972). High threshold and broadly tuned fibres have been recorded in animals with otherwise normal, low threshold and sharply tuned fibres (EVANS, 1972) but such fibres are relatively very few in number. Some evidence however, has been proposed in favour of two populations. PFEIFFER & KIM (1972) for example, found two populations (in the ratio of 93:7) in terms of the change in the number of peaks in post-stimulus time histograms (PSTH) to click stimulation at increasing intensity. However, these observations were only based on fibres with low CFs (below 2 kHz) and cannot be considered to represent the whole cochlear fibre population. These authors also suggested that as the physiological state of their preparation was not adequately monitored, there was a "remote possibilty that the atypical population II response patterns(7%) merely reflected physiological variances". However, against this possibility is the fact that they often found type I & type II fibres in the same animal and at the same CF, and furthermore there was no indication of the two fibre types having significantly different minimum thresholds.

Other evidence for two discrete populations of cochlear afferent fibres has come from ROMAHN (1976) who claimed that 7% of afferent fibres responded best to a stimulus designed to produce a significant radial shearing motion. These 7% did not however show peculiarities of click PSTH. NOMOTO (1977) has also claimed to find, in the monkey, two populations of cochlear fibres

---

[9] The minimum thresholds only vary within 30 dB limits e.g.(KIANG, 1968; EVANS, 1972).

based on their tuning properties. He regards these populations as reflecting the responses of inner and outer hair cell groups. However, it is possible that the two populations he found could be simply a result of pooling his fibre data across frequency; the differences in tuning seem to reflect the differences in tuning between high CF and low CF fibre responses. In any case, the ratio of the two populations he finds, 2:1, does not fit with the 9:1 ratio between inner radial fibres and outer spiral fibres.

If, as seems likely, most cochlear fibre recordings have been made from fibres innervating the IHCs, then with regard to the contribution of the hair cells to those responses at least two alternatives are possible: either the inner hair cell population alone is responsible for low thresholds and sharp tuning found in most recordings (and that the outer hair cell population exists for a different function), or that the OHCs are involved and influence the IHCs or inner radial fibres to produce the typical responses of the auditory nerve.

There is some evidence which indicates that the OHCs may be responsible the transduction of low intensity sounds and this evidence thus favours the second of the above alternatives, that the OHCs in some way influence the IHCs or the inner radial fibres. Some of this evidence is now reviewed; most of it is indirect and circumstantial and some of it is very tenuous and should thus be considered with caution.

It is possible that the OHCs have a mechanical advantage with regard to BM movement; they are more distant than the IHCs from the attachment of the BM to the inner spiral lamina.

The efferent innervation is primarily to the OHCs (KIMURA & WERSÄLL, 1962; IURATO, 1964). Stimulation of the crossed olivo-cochlear bundle (COCB) raises the threshold of cochlear fibre responses (e.g. WIEDERHOLD, 1970; WIEDERHOLD & KIANG, 1970; TEAS et al. 1972). This effect has also been demonstrated in gross CAP recordings (e.g GALAMBOS, 1956; NIEDER & NIEDER, 1970; WIEDERHOLD & KIANG, 1970). These studies suggest that the threshold shift is associated with a change in the response of OHCs. Thus TEAS et al. (1972) pointed out that because efferent COCB stimulation has its major effect at the OHCs, but that the afferent fibres which respond to this stimulation mainly innervate the IHCs, then the "initial stimulating events for nerve fibres at the IHCs must be closely coupled to those at the OHCs." However, although some studies have indicated that the fibres of the COCB lead exclusively to the OHCs (e.g by IURATO, in the rat, 1964), it has been shown more recently by SPOENDLIN (1970) in the cat that some 20% of efferents from the COCB terminate at the IHC afferent synapse. Therefore it cannot be argued with certainty that the effects of the COCB stimulation are mediated by OHCs only.

The first effects of noise overstimulation tend to be at the OHC level, e.g.DAVIS et al. 1937; SCHUKNECHT, 1953; BEAGLEY, 1965; ENGSTRÖM et al. 1966; SPOENDLIN, 1970; ELDREDGE et al. 1973 (hair cell loss) NEUBERT & WÜSTENFELD, 1955; BRUNS, 1976 (hair cell nuclear swelling). Of course, the susceptibility of a hair cell to acoustic overstimulation does not necessarily indicate its threshold sensitivity.

Further evidence for the OHCs having low thresholds comes from numerous studies on the effects of OHC damage or loss on thresholds, both behavioural and electrophysiological. Many of these studies used ototoxic aminoglycosides as a tool for producing the OHC damage. Thus, in the chinchilla, RYAN & DALLOS (1974; DALLOS & RYAN, 1975) used kanamycin to produce selective OHC loss and measured the behavioural thresholds. Normal hearing thresholds corresponded well with intact cochlear regions, and outer hair cell loss resulted in threshold shifts of 30-50 dB.

A most comprehensive correlated study of the behavioural audiogram and cochlear pathology due to ototoxic antibiotics has been carried out in the GP by YLIKOSKI (1974). Kanamycin, gentamicin & neomycin were used to produce hair cell degeneration. Forty two individual experiments were carried out and by pooling data from animals with similar hearing losses, an extremely good correlation between OHC loss and threshold shift was found. "Slight hearing loss was related to moderate loss of OHCs..... The absence of the first row of OHCs was related to a hearing loss of 18 dB; when two inner rows of OHCs were missing, hearing deteriorated by 38 dB, and when all OHCs had degenerated but IHCs were essentially intact, the accompanying hearing loss was 42 dB. When nearly all hair cells were absent hearing loss was 80 dB." These studies argue in favour of the idea that OHCs are involved in the detection of low intensity signals, although the assumptions concerning IHC function (section 1.4d) have to be borne in mind. In this regard, the good correlation between normal hearing thresholds and the presence of undamaged organ of Corti despite, presumably, sub-critical exposure of the hair cells to kanamycin is evidence for supposing that hair cells which resist degeneration are functionally normal.

Some evidence contrary to the idea that the OHCs are essential in low intensity sound detection should be cited here. WARD & DUVALL (1971, 1972) found normal behavioural audiograms for chinchillas, at all frequencies, despite complete loss of OHCs in the first one and a half turns of the cochlea caused by 15 min. exposure to noise at 123 dB SPL. They therefore conclude that IHCs alone may be sufficient to provide normal sensitivity. Similarly, HENDERSON et al. (1974) found behavioural thresholds in chinchilla which returned to normal levels after an initial temporary threshold shift caused by exposure to impulse noise. Histological examination of the cochleas

revealed almost total OHC loss (IHCs intact). However, the OHC damage was not total, and a few residual OHCs could have been sufficient to provide normal behavioral thresholds. This possibility does not apply to the study of WARD & DUVALL in which it was claimed that there was total OHC loss over extensive regions of the cochlea. It may be significant however that WARD has also reported (communication to the authors of BREDBERG & HUNTER-DUVAR, 1975) that one chinchilla with total hair cell loss (OHCs & IHCs) also exibited normal behavioural thresholds at the corresponding frequencies ! It is also worth noting that WARD & DUVALL'S findings have not been confirmed by a repetition of the experiments by other workers (HUNTER-DUVAR & BRELBURG, 1974). In any case it is difficult to reconcile the above mentioned findings with other behavioural studies on hearing loss after noise exposure which showed a good correspondence between threshold elevation and OHC loss (e.g. ELLIOTT & McGEE, 1967; CLARK et al. 1974; DOLAN et al. 1975; HAWKINS et al. 1976).

There are many electrophysiological studies in which CAP thresholds were found to be elevated after OHC loss caused by aminoglycoside poisoning. E.g. DAVIS et al. (1958; using streptomycin) and DALLOS and his co-workers (DALLOS et al. 1972; DALLOS, 1973; DALLOS & WANG, 1974; DALLOS & CHEATHAM, 1976) have found that CAP threshold responses were elevated as a result of OHC loss. DALLOS & WANG (1974) found a shift of 30-40 dB in a cochlea with extensive total OHC loss.

In cochlear fibre studies in kanamycin treated cats, the data of KIANG et al. (1970) indicated a 40-50 dB elevation of the minimum thresholds of cochlear fibres which may have innervated cochlear regions of OHC loss. Figure 1.5 shows the results from one animal in that study. A similar finding with regard to minimum threshold elevation is implicit in a more recent study by DALLOS et al. (1977) in kanamycin treated chinchillas.

In contrast to the above is a study by ROMAHN (1974; ROMAHN & BOERGER, 1977) in which it is claimed that some cochlear fibres from regions of total OHC damage have normal minimum thresholds. This claim will be fully discussed in section 7.0.

Although not entirely unequivocal, much evidence supports the view that the OHCs are essential for the detection of low intensity sounds. How then do afferent cochlear fibres, most of which innervate the IHCs give low threshold responses?

1.3e INTERACTION BETWEEN INNER AND OUTER HAIR CELL SYSTEMS.

It has been suggested that perhaps there is an interaction between afferent fibres of the OHCs and IHCs. However, as mentioned in section 1.2, there is no evidence of a synaptic region between inner radial and outer spiral fibres.

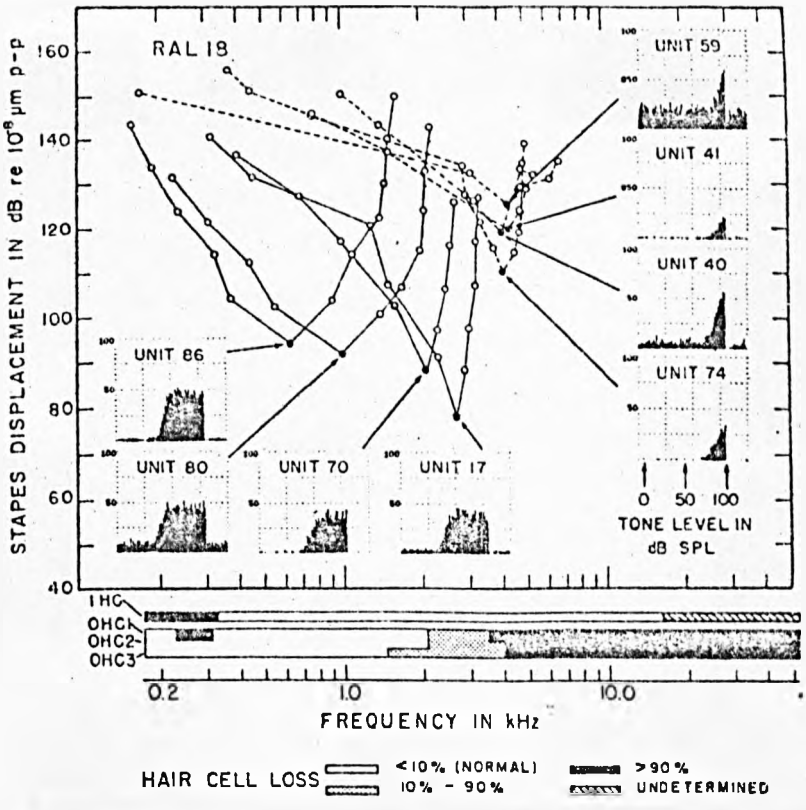


FIGURE 1.5 (from KIANG et al. 1970). Frequency threshold curves (FTCs) and histograms of fibre discharge rate against stimulus intensity for 8 cochlear fibres in a kanamycin treated cat. The shape of the FTCs denoted by the solid lines resemble those of normal cats; the curves denoted by the dotted lines are abnormal. The extent of the cochlear lesion produced by kanamycin poisoning is represented in the cochleogram at the bottom of the figure.

The two types of fibres do, however, run close together (while both are unmyelinated) in the habenula perforata, and this has been suggested as a possible site for electrical interaction (e.g.SMITH & DEMPSEY, 1957; SPOENDLIN, 1966, 1970, 1973, 1974; LYNN & SAYERS, 1970; ARTHUR et al. 1971; EVANS, 1974c, 1975a,b,c; NIEDER, private publication 1974).

SPOENDLIN (1970) drew attention to the change in calibre of all afferents as they passed through the habenula and the possible functional importance of the associated change in axon membrane capacitance as the site of action potential initiation. He suggested that potentials (electrotonically propagated) from a few outer spiral fibres and many inner radial fibres could summate at this initial segment to trigger action potentials.

LYNN & SAYERS (1970) proposed a model[10] which involved a neural interaction at the habenula perforata based on signal propagation in the outer spiral fibres by electrotonic spread. They do not, however, rule out the possibility of action potential propagation. Indeed if such a neural interaction exists at all, action potential propagation would seem more realistic, because the small diameter of these fibres (0.5 µm, SPOENDLIN, 1970) gives an estimated space constant[11] which is rather small compared to the total length of the outer spiral fibre. (EVANS, in a comment on SPOENDLIN (1973) puts its value at approximately 10 µm). The speed of action potential propagation in a 0.5 µm diam. outer spiral fibre could be well over 1.5 m/s [12] (RUSHTON, 1951), giving a propagation time for a 0.5 mm outer spiral fibre of approximately 0.3 ms. If this is added to a synaptic delay time of 0.5 ms (the hair cell synapse) it is not incompatible with the minimum neural latency values of c. 1 ms (KIANG et al. 1965; ANDERSON et al. 1971; EVANS, 1972). NIEDER (1974) considered the possibility of action potentials propagated along outer spiral fibres. He suggested that the constriction at the habenular opening constitutes a high resistance to the conductive path outside each fibre; generator potentials on inner radial fibres are therefore blocked until an action potential along a spiral fibre causes a drop in fibre membrane resistance and a return pathway is provided for the afferent fibre dendritic currents.

---

10 This model was an attempt to account for the shift towards shorter latencies of the centre of gravity of peaks in the click evoked PSTH, as click intensity was increased. This however, may be more satisfactorily accounted for in a model (DUIFHUIS, 1972) in which the response time of the cochlear filter is reduced as a result of reduced "Q" (sharpness of tuning) at high stimulus levels.

11 The length over which voltage across an axon membrane falls to $\frac{1}{e}$ of its original value.

12 It is worth noting that the speed of propagation of action potentials in such unmyelinated fibres is likely to be faster than in a myelinated fibre of the same dimensions (RUSHTON, 1951).

EVANS (1974b, 1975a, b, c) has suggested that the FTC may be generated by two processes related to the inner and outer hair cells such that the OHCs may be responsible for the low threshold, sharply tuned segment of the FTC, and the IHCs the more broadly tuned, high threshold 'tail' segment of the FTC. He has suggested that action potentials propagated along outer spiral fibres could interfere with or initiate discharges in the initial segment of the inner radial fibres where they run closely together (in the habenula perforata).

Another type of neural interaction between the outputs of the inner and outer hair cells has been proposed by ZWISLOCKI (1974, 1977; ZWISLOCKI & SOKOLICH, 1974). This model is based on contributions from the inner and outer hair cells whose differences in sensitivity are within about 10 dB and which are both relatively sharply tuned. The tuning of the contributions supposedly reflects BM mechanical tuning. However, the BM tuning data chosen for the model is that of RHODE (1971) which is anomalous compared with other BM tuning measurements (e.g. JOHNSTONE et al. 1970; WILSON & JOHNSTONE 1972, 1975). In any case, the model requires an inhibition (explicitly synaptic) of the output from the IHCs by the OHCs. Unfortunately no suitable synapses seem to exist.

An interaction between inner and outer hair cells mediated neurally is only one possible mode of interaction. Direct electrical interaction i.e. IHCs being sensitive to potentials generated by the OHCs has been suggested (e.g. GEISLER, 1974; EVANS, 1975a; DALLOS & CHEATHAM, 1976) and models have been proposed on these lines for a cochlear filtering mechanism (MANLEY, 1977; DALLOS & HARRIS, 1977). It will be appropriate to consider these models more fully in the discussion chapter 7.0.

1.4 THE OTOTOXIC ANTIBIOTIC KANAMYCIN.

Kanamycin, an aminoglycoside antibiotic, was the experimental tool used in this study to selectively destroy regions of OHCs, leaving IHCs in those regions apparently intact. In the following sections (1.4a & b) a brief review of its ototoxicity is presented, including the pattern of hair cell damage it causes.

Section 1.4c considers the important question of whether the hair cells remaining in the cochlea after kanamycin poisoning are normal.

1.4a THE OTOTOXIC EFFECTS OF KANAMYCIN.

The most ototoxic antibiotics are those of the aminoglycoside group and include streptomycin, kanamycin, gentamicin and others which are closely

related in their pharmacology and toxicity.

Since the first reports in 1945 (HINSHAW & FELDMAN) on the ototoxic side effects of streptomycin, there has been extensive documentation on how the vestibular and auditory system is affected by streptomycin and subsequently discovered aminoglycosides. Kanamycin, the ototoxic antibiotic used in the present study, was first isolated by UMEZAWA (1958) and its deleterious side-effect on hearing was soon recognized (FROST et al. 1958; LUSTBERG & HAMBERGER, 1959; NAUTON & WARD, 1959; BENITEZ et al. 1962). Most of these clinical reports were of tuberculosis patients to whom kanamycin was administered. Hearing losses were reported ranging from slight high frequency loss to total deafness. The losses in both ears were usually symmetrical. Loudness recruitment[13] was found sometimes (SATALOFF et al. 1964) but not always (HAWKINS, 1959). Histological examinations were possible in some instances (BENITEZ et al. 1964; JØRGENSEN & SCHMIDT, 1962; LOWRY et al. 1973) and these demonstrated that the greatest damage was in the outer hair cells in the basal turn of the cochlea.

1.4b THE PATTERN OF HAIR CELL DEGENERATION.

The sequence of degeneration of cochlear structures caused by kanamycin in experimental animals soon became established. HAWKINS (1959) was one of the first to demonstrate the greater vulnerability of the OHCs in the basal region (in cat and GP). The observations on the effects of kanamycin were very similar to those already found for streptomycin poisoning (e.g. DAVIS et al. 1958). WARD & FERNANDEZ (1961), MESOLELLA & COSTA (1960), BECK & KRAHL (1962), ARDOUEN et al. (1963), FARKASHIDY et al. (1963) made similar histological findings which were extended by HAWKINS & ENGSTRÖM, (1964), KOHONEN, (1965) and ENGSTRÖM et al. (1966) using the surface preparation technique[14] to map out the pattern of hair cell degeneration. These studies using the surface preparation technique demonstrated its usefulness, particularly its advantages over cochlear sectioning methods, although these authors did not extend its use beyond taking small samples from each cochlear turn. YLIKOSKI (1974) and DALLOS & WANG (1974) used the surface preparation technique to survey the whole length of the organ of Corti, and graphically represented the total patt-

---

13 Loudness recruitment is an abnormally rapid increase in the loudness sensation with increase in intensity of an acoustic stimulus.

14 The surface preparation is a histological technique involving microscopic examination of the surface of the organ of Corti (after removal of the tectorial membrane). It was first used by RETZIUS in the 1880's but has only recently gained popularity as a method of assessing cochlear damage. (see section 2.8).

ern of hair cell degeneration in the form of a cochleogram (see section 2.8). Figure 1.6 (from DALLOS & WANG 1974) illustrates twelve such cochleograms from kanamycin treated GPs with different degrees of hair cell loss. These animals were all given a similar regime of kanamycin treatment (400 mg/kg/day for 8-14 days, by subcutaneous injection). The cochleograms in figure 1.6 are arranged to illustrate the sequence which hair cell degeneration follows: OHCs at the basal end of the cochlea degenerate first. OHC degeneration progresses apically and it becomes clear that the first row of OHCs is most affected (e.g.19R, 32L & 13L of figure 1.6). In many instances, complete OHC degeneration has occurred in the basal turn, with little or no IHC degeneration. The OHC degeneration continues, especially in row 1 OHCs, and then IHCs at the apex of the cochlea begin to degenerate. A severe ototoxic insult with kanamycin can result in total degeneration of the sensory epithelium. In any restricted area, sensory cell degeneration precedes that of other, non sensory, structures.

1.4c ARE IHCs NORMAL AFTER OHC LOSS?

In the present study, the adopted criterion for hair cell damage was the presence or absence of cells; mapping out the pattern of cochlear damage by counting the hair cells present or lost was a relatively simple task. To use any criterion of partial hair cell damage would have been difficult with light microscopy. However the possibility exists that hair cells which remain in the cochlea after kanamycin poisoning may look normal (especially under light microscopy), but be partially damaged and functionally abnormal. This point is underlined by a number of biochemical studies on the cochlea immediately after aminoglycoside adminstration which reveal functional, metabolic abnormalities despite normal appearance, e.g. a decrease in succinic dehydrogenase activity (KOIDE et al. 196[7]; GOZDZIK-ZOLNIERKIEWICZ, 1969; KAKU et al. 1973) and a decrease in the uptake of hemicholinium 3 by hair cells (GUTH et al. 1974).

In this present study, and others (e.g DALLOS & WANG, 1974) a period of 2-8 weeks was allowed to elapse after the last injection of kanamycin before electrophysiological recording experiments were carried out. The recovery period was probably long enough to allow kanamycin to be cleared completely from the cochlea. Twenty-four hours after a single kanamycin treatment, its concentration in the inner ear fluid of the GP has usually fallen to about 10% of the maximum concentration reached (VOLDRICH, 1965; WATANABE et al. 1971; STUPP et al. 1973). A similar pattern was observed with related aminoglycoside antibiotics (streptomycin in cat: VRABEC et al. 1965; in man: MEYER ZUM GOTTESBURGE & STUPP, 1969; gentamicin in GP: QUANTE et al. 1974).

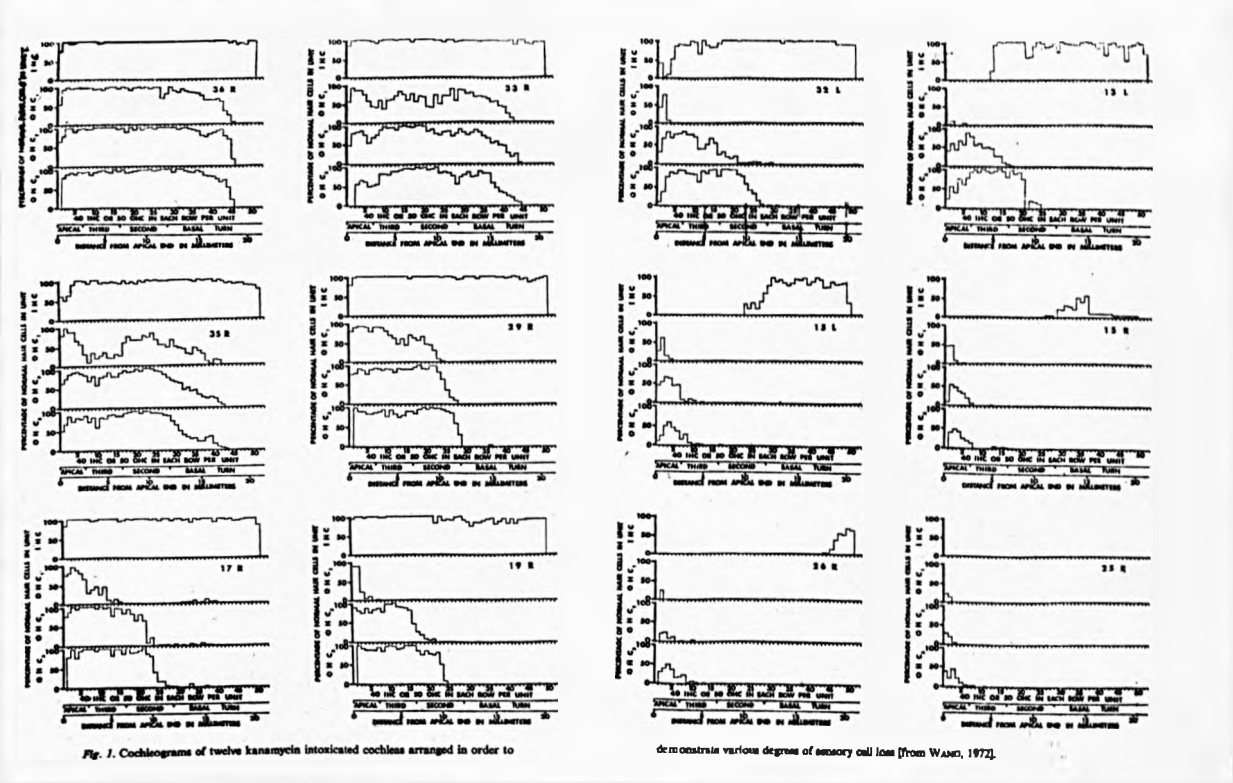


Fig. 1. Cochleograms of twelve kanamycin intoxicated cochleas arranged in order to demonstrate various degrees of sensory cell loss [from WANG, 1972].

FIGURE 1.6 (from DALLOS & WANG, 1974). Cochleograms showing the pattern of hair cell degeneration in 12 kanamycin poisoned GP cochleas, arranged to demonstrate increasing degrees of hair cell loss. Each cochleogram indicates the hair cells remaining in each row, at all positions along the cochlear length. The kanamycin dose regime was 400 mg/Kg/day for 8-10 days, by subcutaneous injection.

In a study by BALOGH et al. (1970) on the clearance of dihydro-streptomycin from GP cochlea there was no demonstrable antibiotic remaining after seven days.

The recovery period is important in that it possibly allows viable hair cells to recover. Furthermore the EM studies of YLIKOSKI (1974) showed that after a waiting period (4 weeks), the IHCs were well preserved in areas where OHCs had completely disappeared, and had very few ultrastructural changes. The afferent nerve endings to such IHCs, as well as the spiral ganglion cells were also of normal appearance. This is often not the case immediately after kanamycin treatment. Under such circumstances, HAWKINS (1977) found changes in the stereocilia the cytoplasmic organelles of IHCs. YLIKOSKI (1974) found swollen afferent nerve endings similar to those seen in noise exposure and ischemia (BEAGLEY, 1965; SPOENDLIN, 1969, 1971). However, even shortly after kanamycin treatment, LUNDQUIST & WERSÄLL (1966) found few abnormalities in cells remaining ("IHCs retained their normal shape although lamellated bodies replace some mitochondria") and where hair cells remained there were no changes noted in afferent or efferent nerve endings.

Thus, the histological studies suggest that providing a recovery period is allowed after kanamycin treatment, the IHCs which remain after OHC loss appear normal. This cannot of course be held to reflect a functional normality of the IHCs but it makes it easier to assume that they are. This assumption will be further discussed (chapter 7.0) in the light of the results from the present study.

The following chapters of this dissertation are arranged as follows:

Chapter 2 describes the experimental methods. Chapters 3,4 & 5 contain the results of this study: chapter 3, the histological observations on the effects of kanamycin poisoning on GP cochleas, chapter 4, the single cochlear fibre responses in the kanamycin treated animals, and chapter 5, the results of investigations on the cochlear action potentials (CAP) of these animals. Chapters 6,7 & 8 contain discussion of the results from chapters 3,4 & 5 respectively.

Some preliminary findings from this study are reported in EVANS & HARRISON, 1976. A more detailed account has been published in HARRISON & EVANS, 1977.

CHAPTER 2

METHODS.

2.1. GENERAL.

2.2. THE USE OF KANAMYCIN TO PRODUCE HAIR CELL DEGENERATION.

2.3. ANAESTHETIC TECHNIQUE.

2.4. THE SURGICAL PREPARATION OF THE ANIMAL AND THE ELECTROPHYSIOLOGICAL RECORDING TECHNIQUES.

2.5. THE SOUND SYSTEM.

2.6. THE RECORDING SYSTEM.

2.7a. GROSS COCHLEAR ACTION POTENTIAL RECORDING PROCEDURES.

2.7b. SINGLE COCHLEAR NERVE FIBRE RESPONSE RECORDING PROCEDURES.

2.8. HISTOLOGICAL EXAMINATION OF HAIR CELL DEGENERATION; THE SURFACE PREPARATION TECHNIQUE.

2.9. THE COCHLEAR FREQUENCY MAP.

2.1 GENERAL.

A total of 65 pigmented GPs were used in the main part of this study, of which 15 served as normal controls.[1] The guinea pig (GP), Cavia cobaya, was chosen as the experimental animal for a number of reasons:

a). It was desired to produce large regions of OHC degeneration leaving IHCs in such regions intact. The available evidence suggested that this could be achieved in the GP (HAWKINS & ENGSTRÖM, 1964; KOHONEN, 1965; ENGSTRÖM et al. 1966; DALLOS & WANG, 1974; YLIKOSKI, 1974).

b). To map out the exact pattern of hair cell degeneration in each cochlea, surface preparation techniques were employed. The GP cochlea, standing proud from the otic capsule into the bulla cavity (see figure 2.6), lends itself well to the microdissection involved in removing the organ of Corti (ENGSTRÖM et al. 1966).

c). A directly measured cochlear frequency map existed for the GP (WILSON & JOHNSTONE, 1972).

The stages of the experimental procedure are outlined below, and the following sections of this chapter describe in detail the experimental techniques of each stage.

Kanamycin (400 mg/kg/day) was administered to GPs by subcutaneous injection for 8-10 days. At least two weeks elapsed after the final injection before each animal was used in an acute electrophysiological experiment. (Section 2.2).

The GP was anaesthetized with a neurolept-anaesthetic technique. (Section 2.3).

Standard surgical preparation and micro-electrode recording techniques were employed to record from single cochlear nerve fibres. Gross recording of cochlear action potentials (CAP) was also carried out at an early stage of each experiment. (Sections 2.4 & 2.7).

Within minutes of the end of the physiological recording, the cochleas were fixed and subsequently examined histologically by light microscopy using the surface preparation technique. The pattern of hair cell degeneration was ascertained for the whole length of the organ of Corti, and recorded in the form of a cochleogram. For comparison of the pattern of hair cell degeneration with the electrophysiological data, the cochleogram was scaled to match frequency abscissa of such data using a cochlear frequency map. (Section 2.8).

---

[1] A further 48 animals were used in preliminary gentamicin and kanamycin tests to find an optimum dose regime for producing the required pattern of degeneration.

2.2 THE USE OF KANAMYCIN TO PRODUCE HAIR CELL DEGENERATION.

Healthy, young, pigmented[2] guinea pigs weighing 150-400g were used. All had a strong Preyers reflex and precautions were taken to ensure that incidence of middle ear infection was low.[3]

A kanamcyin[4] dosage of 400mg/kg/day was adopted (after HAWKINS & ENGSTRÖM, 1964) and daily subcutaneous injections for 8-10 days were administered. DALLOS & WANG (1974) had already proved this dosage regime to be optimal in producing OHC loss[5] in the first two cochlear turns. The injection site was varied daily to maximise drug absorption, and the guinea pigs were weighed daily for calculation of dosage. Typically, a reduction in weight occurred during the kanamycin administration period, and some workers have drawn attention to this (e.g. LUNDQUIST & WERSÄLL, 1966; ARAN & DARROUZET, 1975). In the present study the weight loss seemed related to the age of the animal, so that young, rapidly growing GPs usually suffered only reduction in growth rate while older animals (i.e. almost fully grown) often had severe weight reduction, as did very young animals in poor condition. Loss in weight, or reduction in growth usually stopped soon after kanamycin treatment was finished. However, if the loss in weight was severe, it sometimes continued after the treatment period and eventually caused the death of the animal. If this trend was evident, kanamycin treatment was ended. During the period of low growth rate, the animals often did not eat solid food; vitamin supplement was added to their drinking water in these circumstances.

There was no relationship found between weight changes during kanamycin administration and the amount of damage to hair cells.

During kanamycin treatment, the Preyers reflex[6] of each GP was tested daily.

---

2 Pigmented animals were used because preliminary trials with albino GPs indicated that albinos were more resistant to the ototoxic effects of kanamycin (see section 3.2). Pigmented animals also have a dark stria vascularis which makes the location of cochlear turns easier during microdissection of the cochlea.

3 This requirement eventually led to a breeding programme to prevent the introduction of middle ear disease from foreign stock.

4 Kanamycin produced by WINTHROP (trade name 'Kannasyn') and Kanamycin from BRISTOL laboratories (trade name 'Kantrex') were used.

5 Unless otherwise stated, "OHC loss" refers to the selective loss of OHCs, i.e. with IHCs in such areas remaining apparently intact.

6 The Preyers reflex (or pinna reflex) can give only an approximate indication of changes in hearing threshold. In some instances, for example, the reflex can remain unchanged despite extensive high frequency hearing losses (ANDERSON & WEDENBURG, 1965).

The reflex was usually lost after a few days of treatment. If however, the reflex had not been reduced after eight days, the administration of kanamycin was continued until the reflex deteriorated.

The level of ambient noise in the animal house was low. It is relevant to mention this as there is evidence to suggest that the ototoxic effects of kanamycin is potentiated by noise (DAYAL et al. 1971; KROCHMALSKA, 1974; DAYAL & BAREK, 1975), and so noise levels during kanamycin intoxication may be an important characteristic of the regime used.

After the kanamycin administration, a period of 2-8 weeks was allowed to elapse before electrophysiological recording experiments were carried out. This allowed the GP to recover from the ill effects of any weight loss which may have occurred during kanamycin treatment. More importantly, it is unlikely at this stage that any kanamycin would remain in the cochlea (VOLDRICH, 1960; BALOGH et al. 1970) and the waiting period would also allow damaged sensory cells to degenerate fully, and possibly allow for the recovery of cells only slightly affected. At this stage the critical assumption is being made that hair cells remaining in the cochlea (and of normal microscopic appearance) are functionally normal (see section 1.3c). After the electrophysiological recordings were made, the hair cell degeneration was ascertained and the pattern of hair cell loss was recorded in the form of a cochleogram (see section 2.8). The hair cell degeneration patterns obtained with the kanamycin dosage regime described above are presented in the results section (3.1).

Before the adoption of kanamycin treatment of pigmented animals as the best method of producing selective OHC loss, a number of preliminary histological studies on kanamycin treated albino GPs, had been carried out. The results of these histological examinations are noted in the results section (3.2). It was as a result of these pilot studies that pigmented GPs were used in preference to albino animals.

2.3 ANAESTHETIC TECHNIQUE.

It is often difficult to maintain the GP under stable anaesthesia (e.g. CANNELL, 1972; ROESSLER, 1974; GREEN, 1975). The animal is prone to early respiratory depression, which often precedes a level of analgesia sufficient for surgery. This is especially a problem with commonly used barbiturate anaesthetics such as sodium pentobarbitone. Early cardiovascular depression is also a problem, again especially with barbiturates, making it difficult to maintain a relatively high mean systemic blood pressure (greater than 50 mm Hg) which is essential for normal cochlear function (EVANS 1972).

Because of the unsatisfactory nature of most GP anaesthetic techniques,

experimental trials were carried out in conjunction with Dr. E.F. EVANS, to evaluate a number of anaesthetics which could prove more satisfactory. These were :

a). Sodium thiopentone (Pentothal).

b). Chloralose/urethane mixture.

c). Ketamine.

d). Neuroleptanaesthesia.

The results of these trials, in brief, were:

a). Two trials were performed with sodium thiopentone using an induction dosage of 30 mg/kg intraperitoneally (I.P). In the first trial, this induction dose was administered four times over a period of two hours with little progress towards an anaesthetic state suitable for surgery. In the second trial, this induction dosage was given six times over a one hour period, but respiratory depression occurred before adequate surgical analgesia was produced.

b). One trial was performed with a chloralose/urethane mixture (20 mg/kg and 0.25 g/kg respectively, I.P). Repeated doses at short intervals were required to achieve adequate analgesia. Although early respiratory depression did not occur, the subsequent mean systemic blood pressure of the GP was continuously low (50-60 mm Hg).

c). Three trials were performed with ketamine (44 mg/kg, I.P). Additional pentobarbitone was always required to produce surgical analgesia. A short acting anaesthetic such as ketamine may have been more effective administered intramuscularly but for a small animal such as the GP, this is too traumatic to be an acceptable routine practice.

d). A neuroleptanaesthetic technique for the GP (devised and documented by EVANS, 1978a) was evaluated and used for all the experiments described in this dissertation. It is based on combining neuroleptanalgesic agents, which alone produce (in man) tranquillization and total analgesia, with an hypnotic agent to produce unconsciousness, the technique is thus termed neuroleptanaesthesia.

Neuroleptanalgesia alone has been in veterinary use for many species including GP (GREEN, 1975). The experimental investigations of the present study required the GP to be unconscious, and so minimal doses of barbiturate were administered to produce hypnosis. The protocol was as follows:

The GP was fasted for 12 hours (water allowed). Atropine sulphate (1 mg) was given subcutaneously, followed by an injection of sodium pentobarbitone[7] (30 mg/kg I.P). This is half the induction dose normally recommended for full

---

[7] Freshly made up sodium pentobarbitone (300 mg in 5 ml) was used rather than veterinary solutions because of the possible long term toxic effects of the preservatives (alcohol and propylene glycol) used in the latter. The dosages described are for this freshly made up sodium pentobarbitone solution which is less potent than veterinary solutions (e.g. veterinary Nembutal; Abbot Labs Ltd).

anaesthesia, and after 10-15 minutes it produced hypnosis characterised by a complete loss of muscle tone (and total recumbency). The GP still reacted vigorously to mildly painful stimulation. A neuroleptanalgesic combination was then administered I.P; droperidol[8] (4 mg/kg) was the tranquillizing neuroleptic used, and phenoperidine[9] (1 mg/kg) was the analgesic component. Phenoperidine was preferred to fentanyl (as in veterinary use, GREEN, 1975) because it had a longer lasting effect.

Analgesia developed over approximately 15 minutes and was then usually sufficient for surgery. For more immediate analgesia, intravenous (I.V.) injection of phenoperidine alone was administered.[10] If this failed or was not possible, and the GP was partially analgesic, intramuscular (I.M.) administration of phenoperidine gave total analgesia within a few minutes. Ater the initial induction, complete anaesthesia was maintained for about $1\frac{1}{2}$ hours. Subsequent maintenance doses of phenoperidine (1 mg/kg) were given hourly, via a cannulated jugular vein. The indication for the analgesic was the appearance of a withdrawal reflex elicited by pinching a forepaw. Maintenance of hypnosis was by $1\frac{1}{2}$-2 hourly I.P. injections of 3-6 mg/kg pentobarbitone. Indications were bouts of shivering or sudden elevations of systemic blood pressure and heart rate (see section (2.4) for details on systemic blood pressure monitoring). The pentobarbitone was always administered I.P. thus ensuring a relatively slow uptake into the blood. To encourage even absorption, the pentobarbitone dose was made up to 1 ml in physiological saline. Any cardiovascular depression, which could sometimes occur with considerable delay (10 mins) after I.P. administration, was countered by I.V. or I.A. administration of 5-10 mg of methylamphetamine; such procedures were however rarely necessary.

The neuroleptanaesthetic technique which was developed, provided full and stable anaesthesia with no respiratory depression; artifical respiration was not therefore usually required. Mean arterial blood pressure was maintained at a normal 70-90 mm Hg and this was the important advantage that this technique offered over most others, especially for physiological studies of the cochlea.

---

8 Droperidol is available from JANSSEN Pharmaceuticals Ltd., Marlow, Buckinghamshire. Trade name 'Droleptan'.

9 Phenoperidine is available from JANSSEN Pharmaceuticals Ltd., Marlow, Buckinghamshire. Trade name 'Operidine'.

10 The lateral metatarsal vein was most suitable for percutaneous vein puncture providing there was no skin pigmentation. A xylene rub aided the visibility of the vein.

2.4 THE SURGICAL PREPARATION OF THE ANIMAL AND THE ELECTROPHYSIOLOGICAL RECORDING TECHNIQUES.

The techniques differed little from those established and described by EVANS (1972).

The anaesthetized animal was maintained at 37°c (rectum) with a thermostatically controlled heating blanket.[11] Tracheostomy[12] allowed artificial ventilation[13] if required, and also allowed continuous monitoring of the concentration of end-tidal CO_2.[14] During forced ventilation, this concentration was maintained at 4-5% by adjustment of respiratory pump stroke volume. End-tidal CO_2 concentration was also monitored during spontaneous respiration, the need for artifical respiration was indicated if the concentration of end-tidal CO_2 fell outside normal limits. The right carotid artery was cannulated (1.0 mm o.d. polythene catheter) allowing continuous monitoring of arterial blood pressure.[15] This carotid cannula was kept unblocked by continuous infusion of 3% sodium citrate solution. Permanent records of blood pressure and end-tidal CO_2 concentration were made on a pen recorder.[16] Mean systemic blood pressure was maintained at above 60mm Hg throughout experiments. Peripheral vasoconstriction with methoxamine hydrochloride ('vasoxine') was on rare occasions necessary, as was methylamphetamine for more central cardiovascular depression (e.g. bradycardia). The right external jugular vein was cannulated (1.0 mm o.d polythene catheter) for administration of phenoperidine and other agents as necessary.

Both pinnae were reflected, and external auditory meatae were sectioned leaving them a few mm in length. The animal was mounted in a stereotaxic apparatus[17] with hollow perspex earmoulds replacing the standard ear bars. The cone shaped ear mould was tapered to fit into the stump of the external auditory meatus, and fashioned distally to mate with the condenser earphone (see fig. 2.2). The ear mould also allowed a probe tube to be introduced and positioned

---

11 C.F. PALMER Homeothermic Blanket Control 8142.

12 10 mm o.d. infant endotracheal tube, shortened and fitted to lightweight polythene 'Y' tube for attachment to respiratory pump.

13 C.F. PALMER small animal pump.

14 BECKMAN LBI CO_2 gas analyer.

15 BELL & HOWELL Type 4-442-0001 transducer.

16 GEORGE WASHINGTON 400 MD/2 pen recorder.

17 NARISHIGE (JAPAN).

1 mm from the tympanic membrane, for the purpose of calibrating the sound system (see section 2.5).

The bulla was opened posteriorly and a stainless steel wire electrode (teflon insulated) was introduced to record cochlear potentials from near the round window. The bulla was resealed, but vented to the atmosphere with a nylon tube (0.5mm o.d., 10cm long) thus producing a closed bulla situation, whilst allowing equilibration of pressure each side of the tympanic membrane (the Eustachian tube may be closed). For the intercranial recording from the cochlear nerve, a large, bilateral craniotomy was performed over the posterior fossa. The dura was opened, and the cerebellum and brainstem were deflected medially with a modified metal spatula mounted on a micromanipulator.[17] To free the cerebellum for deflection, a small lobe (the paraflocculus) was aspirated. Constant irrigation with physiological saline prevented any blood accumulating and clotting around the recording site. During manipulation of the cerebellum and brainstem, it was important to avoid compression of the internal auditory artery (labyrinthine artery). The effect of such an occlusion (i.e. cochlear hypoxia) is reflected in a change of threshold for the round window recorded N_1[18] response to a click stimulus. Thus it was essential to monitor this N_1 response continuously during exposure of the nerve, and electrode placement. The N_1 threshold was also checked periodically throughout experiments to monitor any possible acute deterioration of cochlear function.

High impedance (30 MΩ in situ) glass micropipettes were pulled[19] and filled with 3 M KCl. The electrode, mounted on a micro-manipulator,[17] was introduced under direct vision[20] into the cochlear nerve at its postero-dorsal surface, close to the internal auditory meatus. The electrode was inclined at 32° from the horizontal plane and 15-20° from the saggital plane so that it penetrated and travelled in the cochlear nerve, along the internal auditory meatus. The recording site was sometimes stabilized with clear agar,[21] but often this was unnecessary. If the site was not stabilized, it was constantly irrigated to prevent the possible accumulation of blood and the consequent difficulties for further electrode placements. The microelectrode was advanced remotely with a hydraulic micro-manipulator. Micrometer readings indicated the depth of the electrode in the nerve. The electrode impedance was periodically measured in situ by introducing a small constant current pulse to the electrode, creating (across the high resistance of the electrode) a voltage change which was proportional to the electrode resistance. By comparison of this voltage to

---

18 The N_1 is the first negative peak of the compound action potential recorded from the cochlea.

19 PALMER Microelectrode Puller.

20 Using a ZEISS Binocular operating microscope.

21 2g in 100 ml saline.

that produced across a calibration resistor (10MΩ), an absolute measure of the electrode impedance was obtained.

2.5 THE SOUND SYSTEM.

The block diagram of figure 2.1 summarizes the components of the sound system. Pure tones were produced from a low distortion A.F. oscillator[22] and could be trapezoidally gated. The tone frequency was monitored accurately with a digital frequency counter.[23] The rise and fall times, and the duration of the trapezoid modulating wave form could be manipulated. A white noise signal could be gated in a similar manner to that of the pure tone stimuli. A pulse generator of variable pulse width was used to produce a click stimulus. For all signals,[24] the repetition rate and delay (with reference to a synchronizing pulse to trigger, for example, the oscilloscope trace) was controlled from a clock pulse generator with variable delay. A Brüel & Kjaer ½ inch condenser microphone cartridge[25] was used as a transducer, giving an audio output with a frequency range adequate for the GP hearing range (up to approximately 40 kHz). For low sound intensities (up to 70-80 dB SPL) the signal[26] was fed directly to the condenser 'earphone' via a 100 dB step attenuator[27] (1 dB steps). For sounds of higher intensity (greater than 70-80 dB SPL) the signal was amplified with a low noise, power amplifier. At such high sound intensities, i.e. 80-120 dB SPL, the condenser earphone introduces considerable harmonic distortion. The signal from the attenuator to the power amplifier was therefore passed through a distortion compensating circuit which introduced a level dependent distortion to compensate for that produced by the condenser earphone (circuit design by J.P.WILSON, see EVANS, 1978b)

Figure 2.3 shows some example energy spectra of tone signals at maximum sound pressure levels (110-120 dB SPL), which is the worst condition for the production of harmonic distortion. The top diagram shows the harmonic distortion in a gated 1 kHz signal (30 ms duration, 10 ms rise-fall times). The lower diagram shows that of a continuous 1 kHz tone signal. It can be seen that the harmonic distortion in the dynamic situation (i.e gated tones with 10 ms rise-fall times) is more than 49 dB below the level of the fundamental.

---

22 LEVELL R C Oscillator Type T G 150m.

23 Advance timer counter 503.

24 FARNELL pulse generating system.

25 BRÜEL & KJAER type 4134.

26 Harmonic distortion - 70 dB.

27 HEWLETT - PACKARD 350 D.

28 This 'anti-distortion unit' was adjusted to produce minimum harmonic distortion in dynamic signals (i.e gated tones with 10 ms rise fall times) at the maximum sound intensity level.

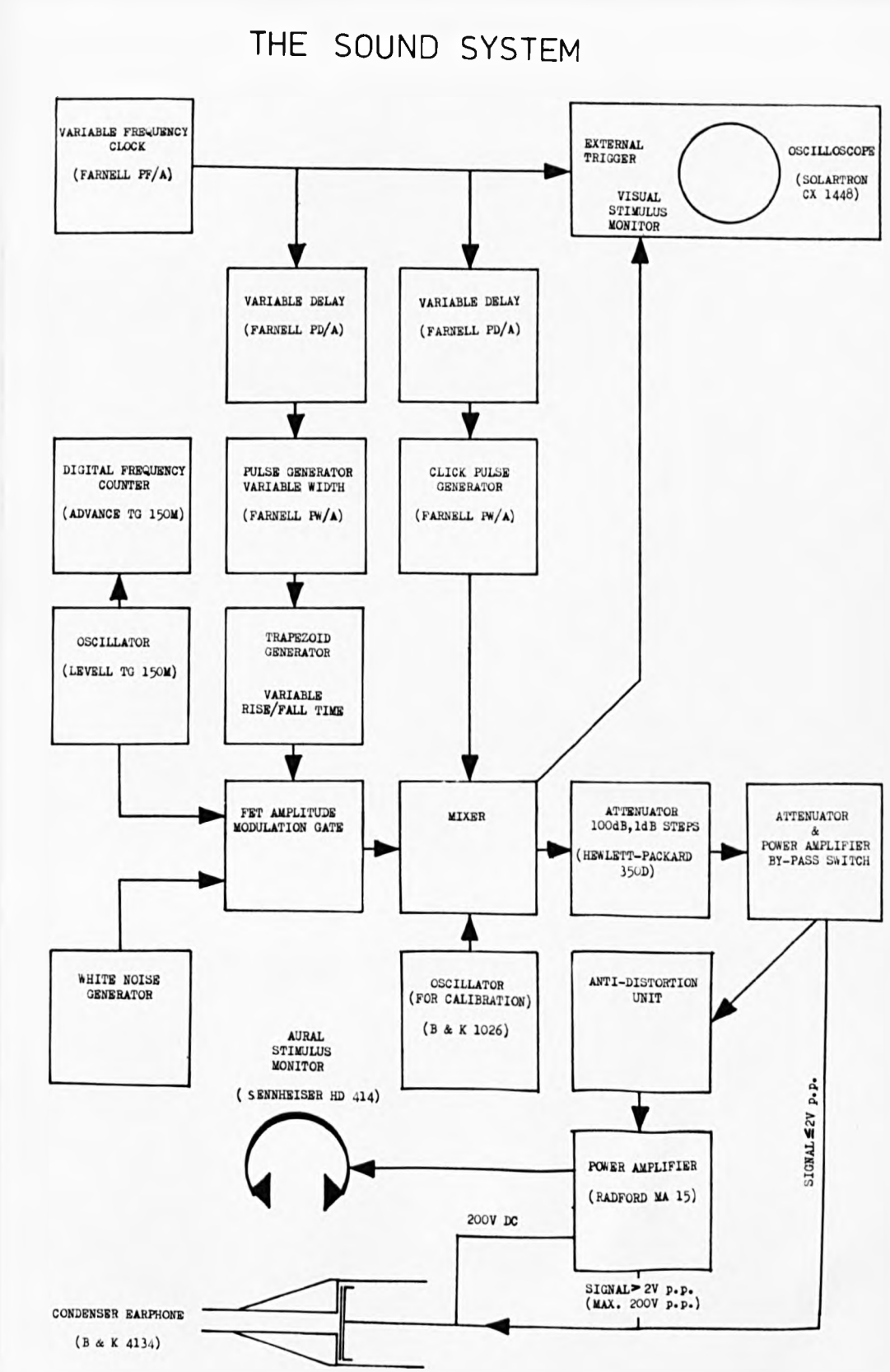


Figure 2.1 Schematic diagram showing the salient features of the sound system used in the present study.

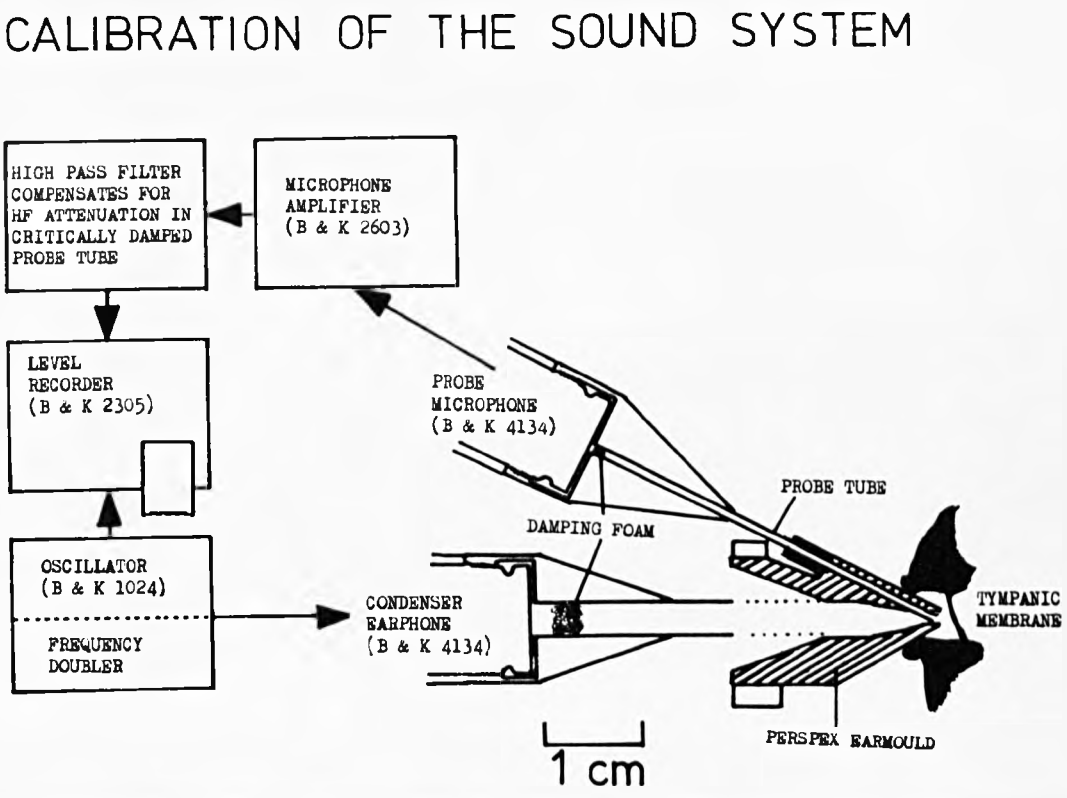


Figure 2.2 The right hand diagram, drawn to scale, shows the postition of the condenser earphone and the probe microphone in relation to the hollow, perspex earmould and the external auditory meatus.

Also shown (schematic; left) is the arrangement of the apparatus for the calibration of the sound system. (see text for details).

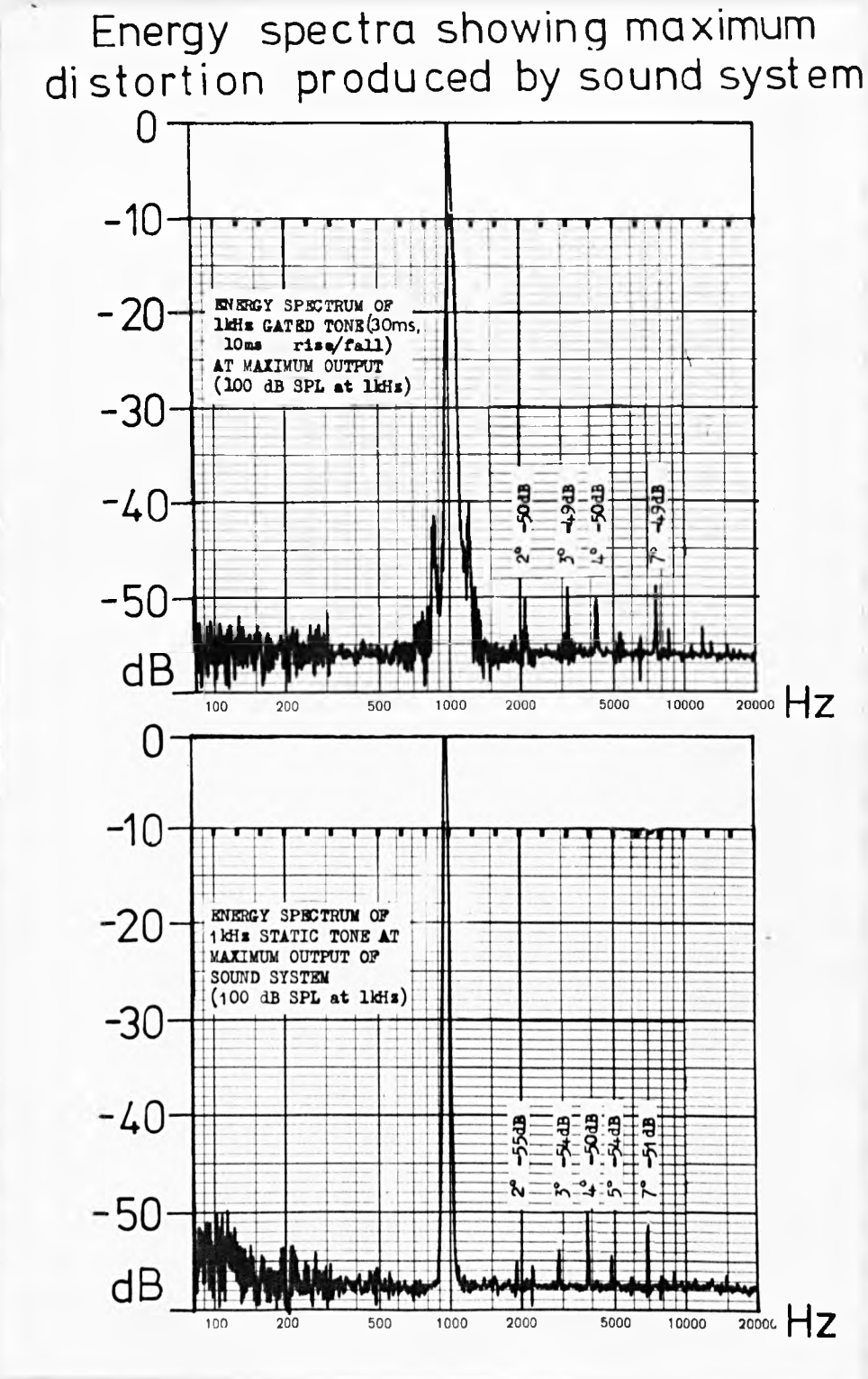


Figure 2.3 Typical energy spectra of tone stimuli used in this study. These energy spectra illustrate the worst conditions of distortion, that is, with the sound output at maximum level (100-120 dB SPL, corresponding to 200 V p.p. input to the condenser earphone). The upper diagram shows the spectrum of a trapezoidally gated, 1 kHz tone (30 ms duration, 10 ms rise/fall times). The lower diagram shows the spectrum of a 1 kHz continuous tone. The distortion shown in these examples is typical.

The power amplifier was modified to provide the 200 V d.c. polarization voltage for the condenser earphone. The earphone was coupled to the perspex earmould via a lightly damped Brüel & Kjaer probe cone and tube (20 mm long 4 mm o.d). The distance from the condenser earphone diaphragm to the tympanic membrane was 3 cm (see figure 2.2). The driver probe tube was damped with polyurethane foam crumbs, and an adjustment of the damping was made to obtain critical damping. The condenser earphone driving system (EVANS, 1978b) produced a maximum stimulus intensity of 110-120 dB SPL (re 0.0002 dyne/cm^2) depending on frequency, for an input signal of 200 V peak to peak.

A Brüel & Kjaer microphone[29] with a 1mm o.d. damped probe tube[30] was inserted into the perspex earmould so that the end of the probe was 1-2mm from the tympanic membrane. For each experimental animal, the sound level at the tympanic membrane was determined[31] via the probe tube, at frequencies from 0.2-40 kHz. The actual sound pressure level at any frequency was calculated from the value of the recorded sound pressure level, adjusted to allow for the attenuation of the probe tube and probe tube microphone at that frequency; the attenuation characteristics of the probe tube were regularly calibrated. Fig. 2.2 summarizes the essential features of the sound calibration system.

The electrical signal producing the sound stimulus was monitored visually on an oscilloscope[32] and also aurally through high fidelity headphones.[33] The oscilloscope was triggered from the main clock pulse generator.

2.6 THE RECORDING SYSTEM.

The recording system is shown diagrammatically in figure 2.4. Round window electrode potentials were pre-amplified[34] conventionally (x1000) before further amplification and display on the oscilloscope. The pre-amplifier passband was usually .08 kHz - 10 kHz. When it was necessary, the gross cochlear action potentials (CAP) were averaged[35] and plotted out on an XY plotter[36]

---

29 BRÜEL & KJAER type of 4134.

30 When this probe tube is critically damped, its frequency response falls off at high frequencies. To compensate for this attenuation at high frequencies, a high pass filter is used in conjunction with the probe tube microphone amplifier. This high pass filter has a cut-off slope designed to compensate as nearly as possible for the cut-off slope of the probe tube frequency response.

31 Via BRÜEL & KJAER microphone amplifier type 2603 on BRUEL & KJAER level recorder type 2305.

32 SOLARTRON CX 1448.

33 SENNHEISER HD 414.

34 TEKTRONIX FM122 pre-amplifier.

35 NUCLEAR CHICAGO dataretrieval computer.

36 BRYANS X Y plotter.

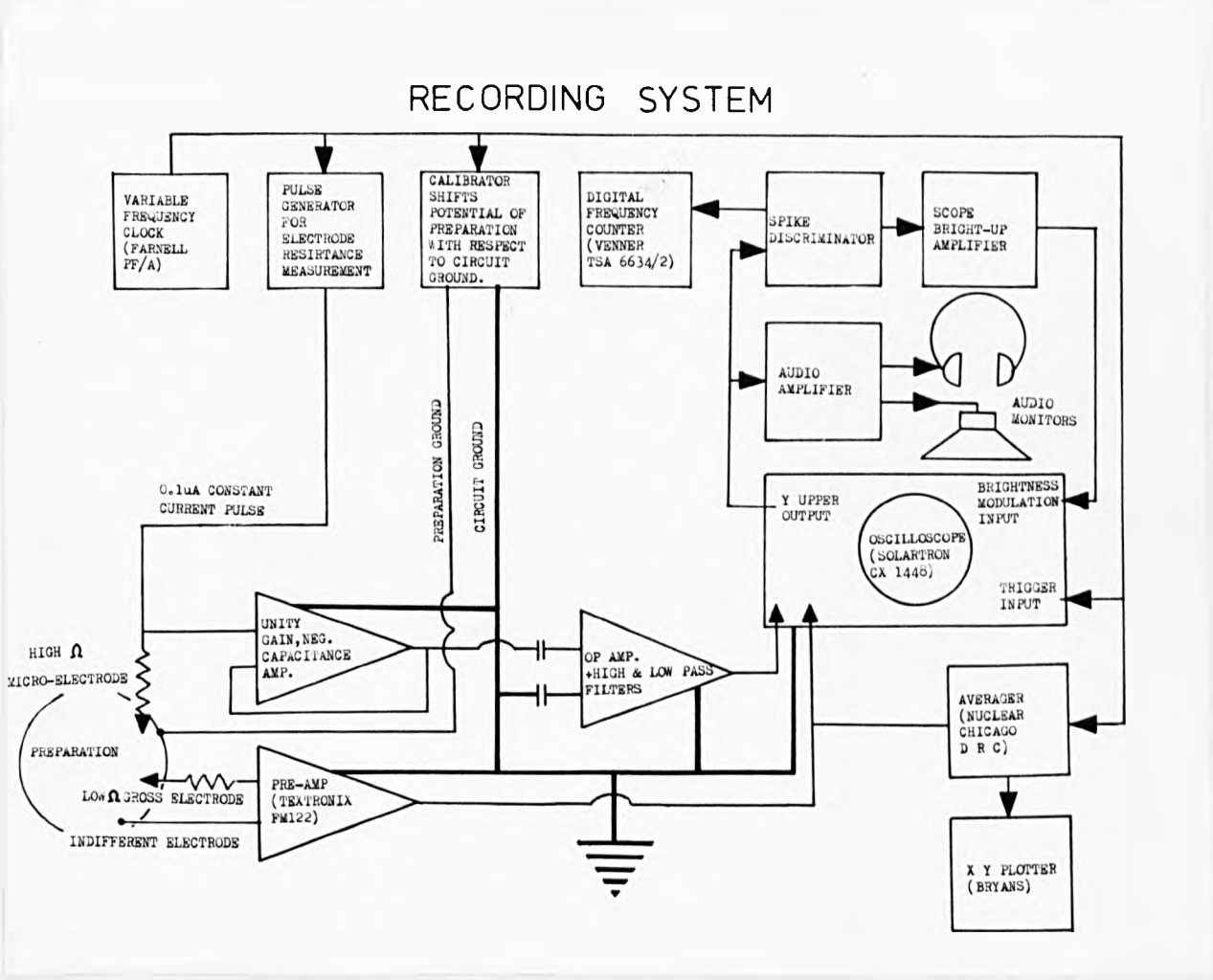


Figure 2.4 Schematic diagram of the electrophysiological recording system. (see text for details).

for a permanent record.

Micro-electrode potentials were amplified via a high input impedance,[37] unity gain amplifier.[38] The effects of stray capacitance particularly that produced across the wall of the micro-electrode was neutralized by negative capacitance feedback. Before display on the oscilloscope, the signals passed via a x100 amplifier, and low and high pass filters. Recordings were usually carried out with a passband of 0.2 kHz - 5 kHz.

Signals from the Y upper amplifier of the oscilloscope were passed through a variable threshold Schmitt trigger so that the peaks of signals, such as action potentials, could be discriminated. The discrimination threshold was indicated on the oscilloscope by brightness modulation of the trace; (a 50V D.C. bright up voltage was gated by the Schmitt trigger output).

Recorded potentials were monitored aurally before or after discrimination. Discriminated spike discharge rates were counted with a digital frequency counter.[39] The preparation ground was connected to the circuit ground via a low source impedance pulse generator. The potential of the preparation could therefore be shifted (50 μv - 100 mv) with respect to circuit ground, and thus provided a means for both the calibration of recorded potentials and a test for electrode contact.

2.7a GROSS COCHLEAR ACTION POTENTIAL RECORDING PROCEDURES.

The N_1 component of the gross cochlear action potential (CAP) evoked by a click stimulus[40] was used to monitor the approximate threshold of response of the cochlea. During microelectrode placement, the CAP was continuously monitored, and the peak of the N_1 was discriminated. This discriminated response was played over a loudspeaker so that any decrease in the N_1 amplitude such that it was no longer discriminated (and no longer audible) served as an immediate warning of cochlear hypoxia. The threshold of the N_1 was also measured periodically during cochlear fibre recording to detect any slow deterioration of cochlear function which sometimes occurred during the experimental proceedings.

For the determination of amplitude and latency functions, 50-200 responses were averaged and plotted out at each of a number of intensity levels (5 or 10 dB steps).

---

37 Input resistance greater than 10 MΩ.

38 Based on ELSA - 4 Bak amplifier.

39 VENNER digital counter TSA 0034/2.

40 Unless otherwise stated a 50 μs rarefaction click was used.

Frequency specific stimuli were used to determine the threshold of the CAP. The stimuli used were tone pips of 4 ms duration, with 2 ms rise-fall times and a repetition of 5/s. Figure 2.5 shows the energy spectra of such tone pip stimuli at frequencies of 1, 4 and 16 kHz. These signals are impulsive enough to evoke a CAP, yet retain good frequency specificity such that near threshold, the CAP response to such stimuli is reflecting synchronous activity in the fibres from cochlear regions appropriate to the stimulus frequency.

The threshold of the CAP to such stimuli were measured by visual detection of the CAP on the oscilloscope trace. Such thresholds were determined at 10-20 frequencies from 0.5 kHz to 40 kHz, thus obtaining the 'CAP audiogram'. A more detailed methodological consideration of the CAP audiogram determination is given in section 8.1.

2.7b SINGLE COCHLEAR NERVE FIBRE RESPONSE: RECORDING PROCEDURES.

Broad-band noise bursts (50 ms duration, 5-10/s) were used as a search stimulus. For cochleas with anticipated high thresholds i.e in kanamycin treated animals, the search stimulus was of sufficient intensity to stimulate any high threshold, pathological fibres. High intensity stimulation was, however, used judiciously to prevent any temporary threshold shift. On encountering a cochlear fibre response, the depth of the electrode in the nerve track was recorded.

The response of the fibre was displayed visually on the oscilloscope, and monitored aurally after spike discrimination. Gated tonal stimuli of 50 ms duration, 5 or 10 ms rise-fall times, and at a repetition rate of 5-10/s were used to determine the minimum threshold and FTC of the cochlear fibre, by the procedure outlined below.

The minimum threshold of a cochlear fibre response was determined by manually sweeping the stimulus across frequency at successively lower intensity levels until the lowest threshold of response was reached. The stimulus frequency at this minimum threshold is the characteristic frequency (CF) of the fibre. The FTC was determined by varying the stimulus frequency towards the CF from high and low frequency sides alternately and at 5 or 10 dB steps until the fibres threshold of response was reached. For all threshold determinations, an audio-visual detection (mainly auditory cues) of a change in firing rate above spontaneous activity was the threshold criterion used.

At the threshold of response, the just detectable rhythm of discharge corresponding to the stimulus repetition rate was easily identifiable. To determine an FTC, as set out above, took approximately 4 minutes.

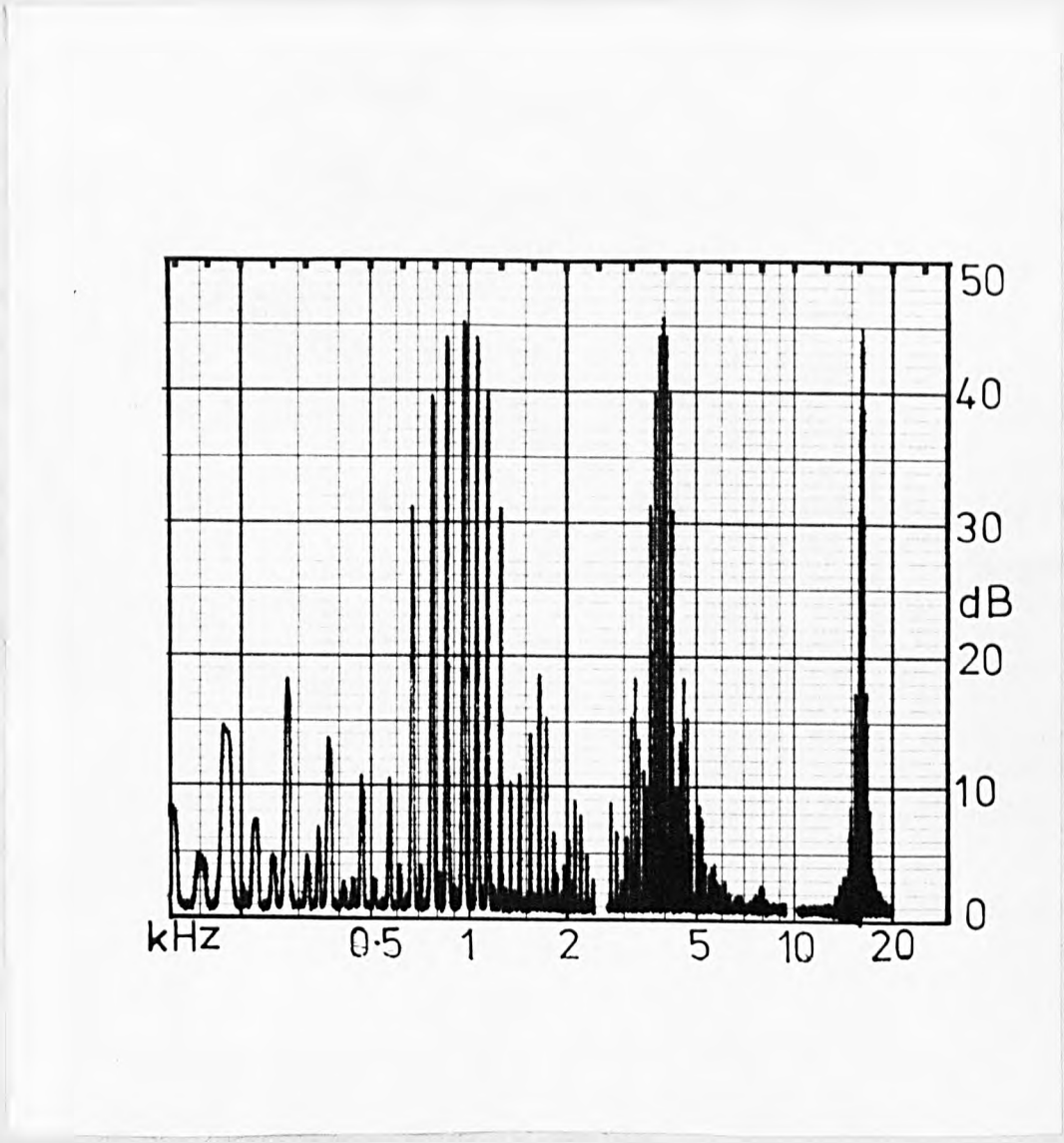


Figure 2.5 Energy spectra of the tone pip stimuli used to evoke frequency specific CAP responses. The examples shown are of tone pips at 1, 4 and 16 kHz, (4 ms duration, 2 ms rise-fall time, repeated at 100/s).

Spike counts of spontaneous activity were then sampled for at least 20 sec, and this was often repeated a minute later.

After the experiment the threshold intensity values were corrected to dB SPL re. 0.0002 dyne/cm^2. Correction was done using the sound system calibration curves of dB SPL at the tympanic membrane recorded at the beginning of each experiment. These calibration curves were corrected for the attenuation characteristics of the probe tube and probe tube microphone used in the ear calibration. For convenience, computer programs written by E.F. EVANS were used for the above correction procedures as well as for calculating bandwidth and slope values of tuning characteristics, and for plotting corrected FTCs. In addition to the manual determination of FTCs described above, semiautomatic and automatic FTC determinations have been made in five animals. The system enabling computer control of stimulus frequency and intensity in the fully automatic determinations was developed by E.F. EVANS, and is described in the appropriate results section.

The results of single cochlear fibre recording experiments are reported in chapter 4 and discussed in chapter 7.

2.8 HISTOLOGICAL EXAMINATION OF HAIR CELL DEGENERATION; THE SURFACE PREPARATION TECHNIQUE.

Immediately after electrophysiological recording, the GP was sacrificed with a barbiturate over-dose, and within minutes post-mortem the cochleas were fixed with 2% veronal buffered osmium tetroxide solution. The quickest routine for fixing the cochleas was to decapitate the GP, remove its lower jaw and open the ventral surface of both bullae, thus exposing the cochleas. As figure 2.6 b & c illustrate, holes were picked in the apex of each cochlea, and near the round window into the scala tympani; the stapes footplate was also punctured. The fixative was allowed to run down through the cochleas from the apex to the base, and when the perilymph was totally replaced by the fixative, the cochleas were removed and immersed in cold (5°c) fixative for an hour. After washing in distilled water, they were stored in 50% alcohol.

The surface preparation technique, as described by ENGSTRÖM et al. (1966) was used to examine the organ of Corti. A clamp on a rotating stage was used to hold the otic capsule firmly, under water, for dissection. All dissection was done under a binocular microscope[41]. Starting at the apex, the thin bony capsule was gradually removed from the cochlea; the stria vascularis and spiral ligament were also removed (see figure 2.6 c & d). Throughout the cochlea, these latter structures could be easily freed from the rest of the

---

[41] ZEISS binocular operating microscope. Magnification x6 to x40.

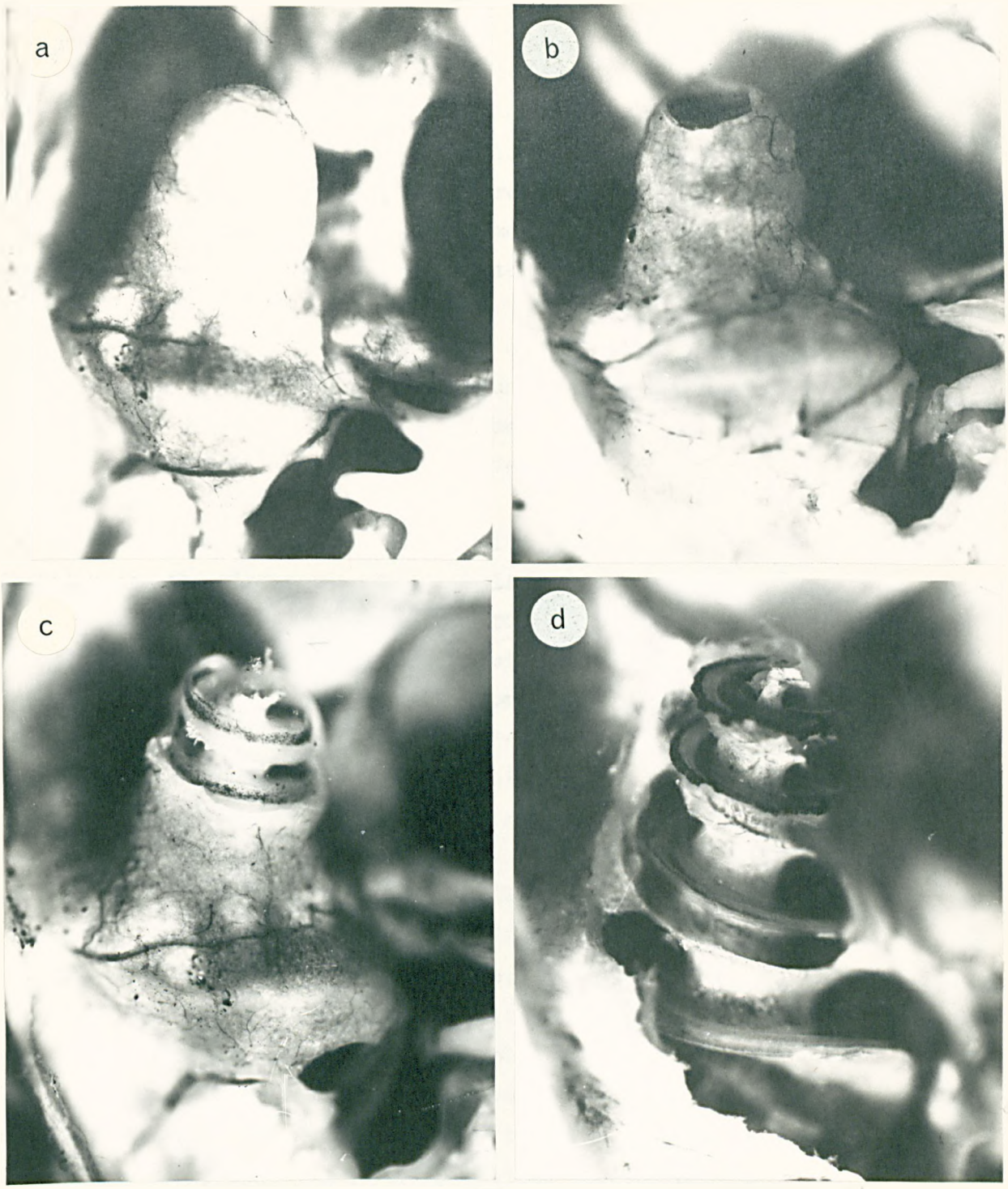


Figure 2.6

(a). The cochlea exposed by removal of the ventral wall of the bulla.

(b). The extreme apical and basal regions of the cochlea opened to allow fixative (2% OsO_4) to flow through the scalae. (the basal opening is more clearly seen in photograph C).

(c). After fixation: the bony shell of the cochlea removed from two apical turns to reveal the spiral ligament and the stria vascularis.

(d). The dissected cochlea, with the bony shell, spiral ligament, and stria vascularis removed from all turns exposing the organ of Corti. (The black border to the organ of Corti in the apical turns is a region of Hensen cells which are osmiophilic and thus darkly stained.

organ because of a line of weakness in the area of the Claudius cells. The organ of Corti was removed in up to 3 mm lengths and was best preserved if still attached to some bony spiral lamina by which it could be handled safely. (The thickness of too much spiral lamina could, however, interfere with high power microscopy which requires short objective working distances). The basal turn of the cochlea was the most difficult to dissect, but the organ of Corti tended to attach very firmly to the bony spiral lamina and was rarely damaged (after practice). The extreme basal 'hook' region was sometimes lost, but never without accurate measurement of the distance over which the loss occurred. Each surface specimen was mounted in an aqueous mounting medium[42] for light microscopic examination. For some (early) parts of this study, phase contrast optics were used.[43] Later, differential interference microscopy was adopted.[44 45]

The length of each surface specimen was measured (along the tunnel of Corti) under the microscope, using a calibrated eyepiece graticule; the measurement error for each specimen was approximately ± 0.02mm. The examination of the specimens was usually carried out under magnifications of X400-X1000 (using oil immersion objectives). Despite the thickness of the tissue, much detail could be seen, especially with 'optical sectioning' under differential interference microscopy. The orderly arrangement of the hair cells at the cuticular lamina made the detection of hair cell loss relatively easy. This neat pattern of the surface of the organ of Corti is illustrated in figures 2.7 & 2.8 which show medium and high power photomicrographs of normal regions of the GP cochlea. Where there was a disruption of the cuticular lamina, the task of assessing hair cell loss was more difficult. For example, in areas of kanamycin treated cochleas where the first row of OHCs had degenerated completely, the remaining OHC rows often collapsed into the resulting gap so that OHC row 2 ambiguously appeared as OHC row 1. There was sometimes difficulty in observing row 3 OHCs at the apex of the cochlea because such cells were often obscured by the large, darkly stained osmiophilic inclusions of the Hensen cells in this region.

The pattern of hair cell damage was ascertained by logging the hair cells remaining in each hair cell row. The simple criterion of presence or

---

42 e.g. Farrants aqueous mounting medium (GURR); GURR 'uvinert' aqueous mountant.

43 NIKON L-Ke with phase contrast.

44 NIKON L-Ke with differential interference.

45 ZEISS (W.Germany) standard 14 with Nomarski differential interference contrast.

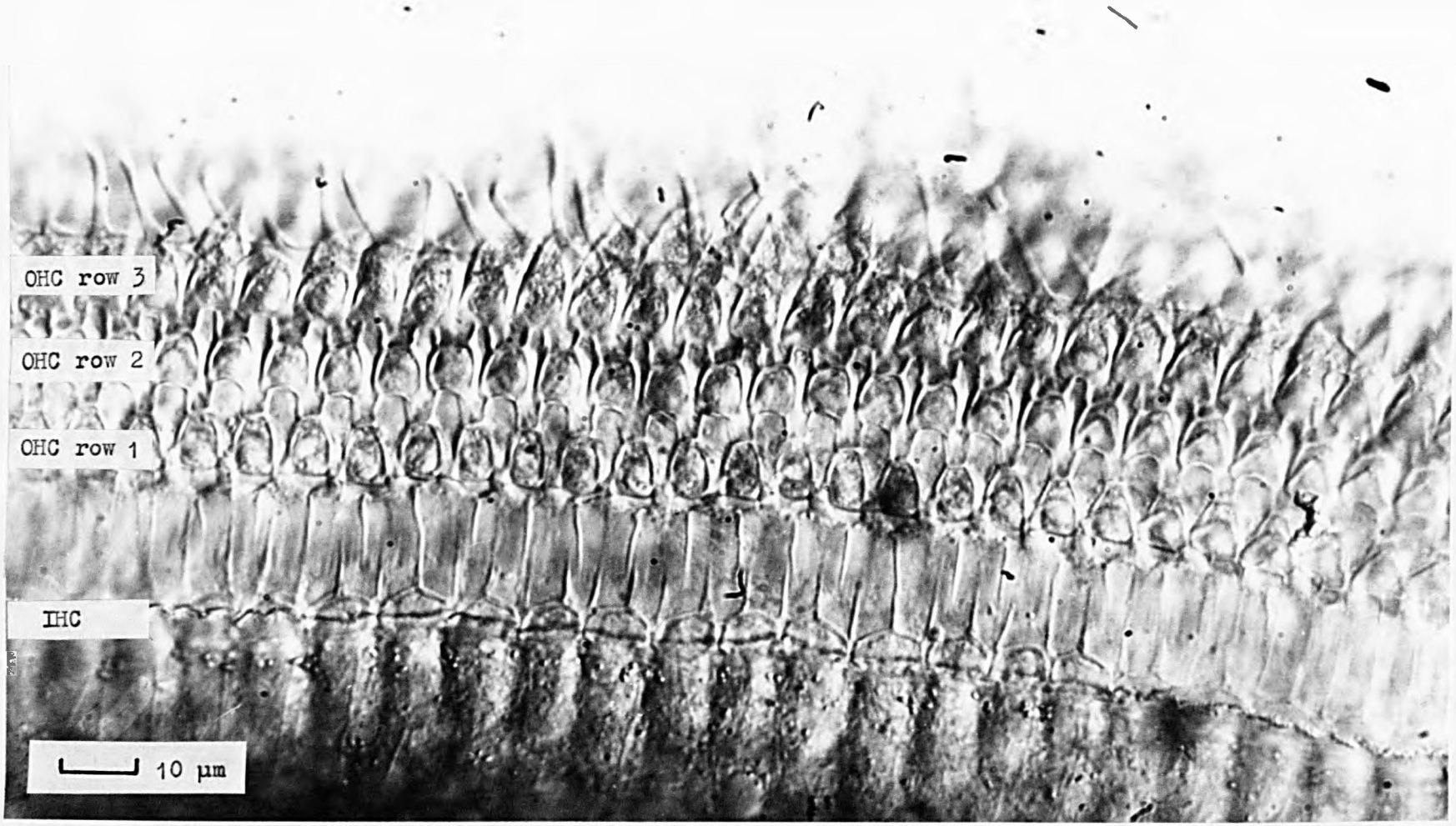


Figure 2.7 Photomicrograph of the upper surface of the organ of Corti from a normal cochlea. The microscope optics were Nomarski differential interference - contrast.

Figure 2.8 Photomicrograph, taken at high magnification, of the surface of the organ of Corti from a normal cochlea. Near the surface of each hair cell, the (optical) cross section through the bases of the stereocilia reveals their 'W' shaped arrangement. The microscope optics were Nomarski differential interference-contrast.

absence of hair cells was the only criterion which could be practically applied. Indeed no hair cells remaining (more than two weeks) after kanamycin poisoning were found to have any partial damage which could have been used as a criterion of hair cell damage (e.g. abnormal cytoplasmic granulation, loss of stereocilia). This was not unexpected because even at the electron-microscopic level, there are no morphological abnormalities in hair cells remaining four weeks after kanamycin poisoning (YLIKOSKI, 1974).

From the comprehensive log of the hair cell counts, cochleograms were constructed. The format for the cochleogram was based on that of DALLOS & WANG (1974). See for example figure 4.10: the percentage of hair cells present were plotted for "bin" widths of 50 cells for the three row of OHCs, and 40 cells for the IHC row. The ratio of the IHCs to OHCs changes insignificantly along the length of the GP cochlea (COLEMAN, 1975). Supernumerary hair cells (e.g. row four OHCs) are not indicated on the cochleogram.

The length of the cochlea was expressed in mm from the basal extreme of the organ of Corti. The error in estimation of total length was 0.2mm to 0.5 mm depending on whether the whole spiral organ was removed successfully and measured microscopically, or whether the length of a lost specimen had to be inferred from the gap in the spiral lamina measured under the low power dissecting microscope. The results of these histological studies on hair cell degeneration caused by kanamycin are presented in chapter 3, and discussed in chapter 6.

2.9 THE COCHLEAR FREQUENCY MAP.

To allow correlations to be made between the electrophysiological data and hair cell losses, the cochlear frequency map of WILSON & JOHNSTONE (1972) was used. This map is shown in figure 2.9. The map was derived, at high frequencies, from direct measurements of basilar membrane peak amplitude of vibration by WILSON & JOHNSTONE, and at low frequencies, from the cochlear microphonic data of DALLOS.[46]

This cochlear frequency map gives a high frequency limit of 43 kHz which is consistent with the behavioural audiogram of the GP (HEFFNER et al. 1971). The low frequency data points of DALLOS were derived from a cochlea which was 22 mm in length. The apical extreme of such a cochlea has, according to the map, a low frequency limit of 0.08 kHz, very close to the CF of the lowest frequency cochlear fibre found in this study. Differences in the length of individual cochleas, from the 22 mm of the frequency map were accommodated by linear scaling (see figure 6.2).

The need for an accurate cochlear frequency map for correlating hair cell loss to the electrophysiological data was emphasized in the introduction

---

[46] DALLOS (1971) J. Acoust. Soc. Amer., 49, p 1141, figs 1 & 2; p 1820, fig. 1.

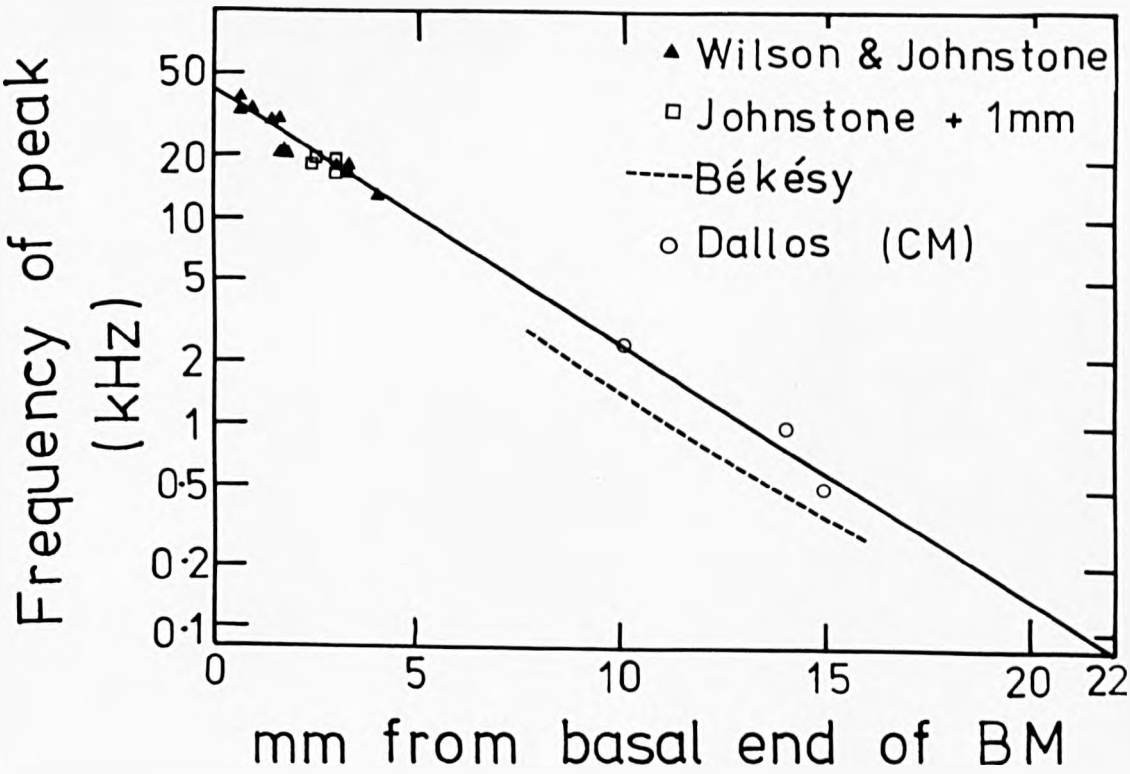


Figure 2.9 (After WILSON & JOHNSTONE, 1972). The cochlear frequency map used in the present study. The filled symbols are those of WILSON & JOHNSTONE and indicate the frequency of peak BM responses as a function of distance from the base of the cochlea. Also shown are the points of JOHNSTONE et al. (1970 open squares) measured from the stapes (to which 1 mm has been added), the data of BÉKÉSY (dashed curve), and the cochlear microphonic data from DALLOS (1971).

(section 1.1a). Further confirmation of the accuracy of the map used was found from the results of the present study. These findings and those of others which support the accuracy of the frequency map are discussed in section 6.4.

CHAPTER 3.

RESULTS: HAIR CELL DEGENERATION.

3.1 THE PATTERN OF HAIR CELL DAMAGE PRODUCED BY KANAMYCIN POISONING.

3.2 THE OTOTOXIC EFFECTS OF KANAMYCIN ON ALBINO GUINEA PIGS.

3.3 HAIR CELL DAMAGE AFTER LONG TERM SURVIVAL FROM KANAMYCIN TREATMENT.

3.1 THE PATTERN OF HAIR CELL DEGENERATION PRODUCED BY KANAMYCIN POISONING.

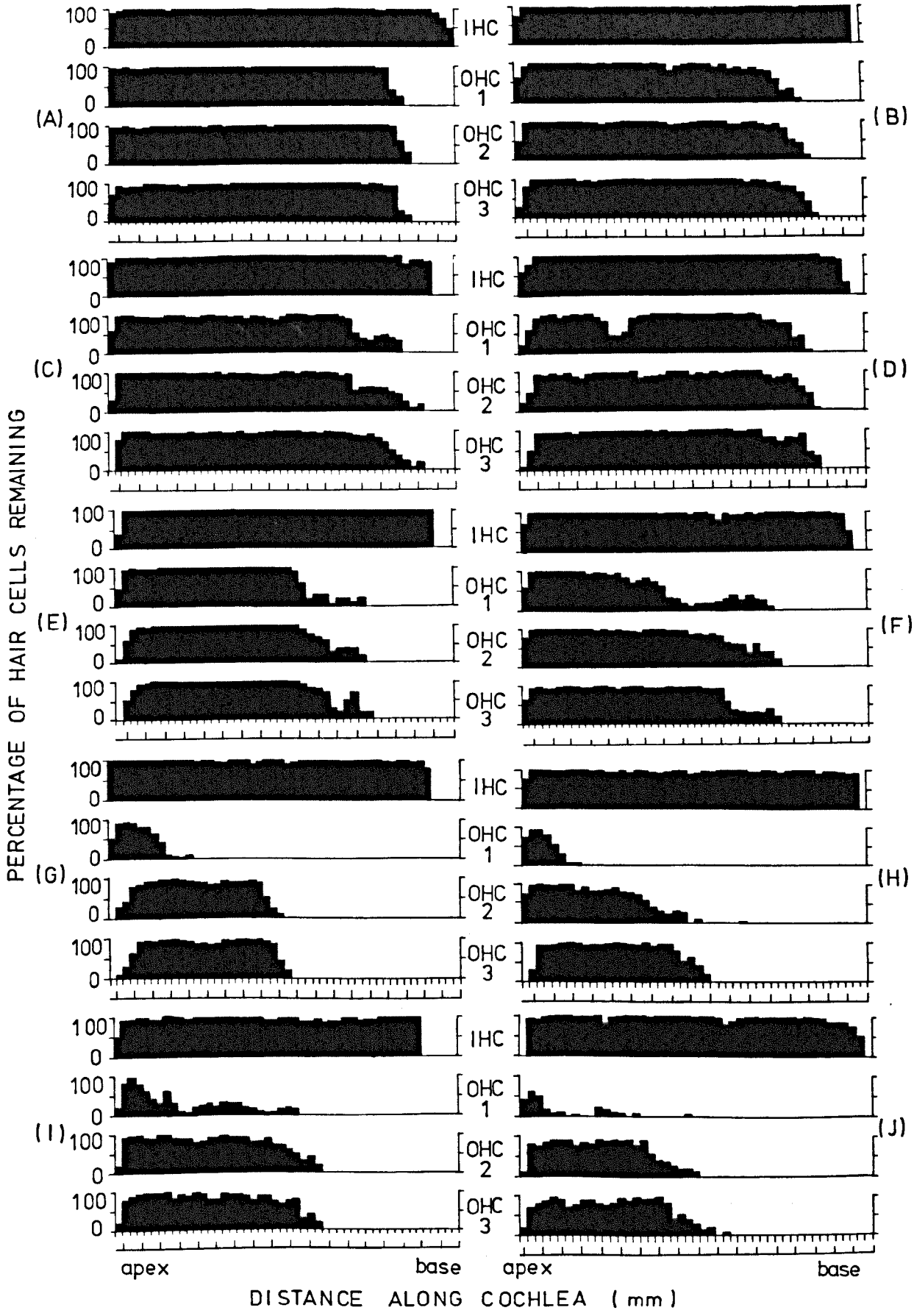
Figures 3.1 a & b show a sample of cochleograms illustrating the range of susceptibility between animals to similar dosages of kanamycin. All the cochleograms shown are of the left[1] cochlea of GPs given 400 mg of kanamycin/kg/day for 8-10 consecutive days (by sub-cutaneous injection). The animals were sacrificed between 2-10 weeks after the kanamycin treatment. The damage ranged from very little basal OHC loss (cochleogram A) to total hair cell degeneration (cochleogram T). Figures 3.1 a & b also qualitatively illustrate the sequence in which kanamycin damages the hair cells. The OHCs at the basal, high frequency region of the cochlea degenerate first, and this OHC loss gradually extends more apically. The first row of OHCs seems to be more susceptible than rows 2 and 3 (e.g. cochleograms J - O). At a very late stage in the sequence of degeneration (i.e. when most OHC have degenerated), the IHCs start to be damaged, sometimes at the apical end of the cochlea (Q,R) and sometimes at the basal end (N,O).

Figures 3.2 - 3.4 show photomicrographs of surface preparation specimens of organ of Corti, partially damaged by kanamycin poisoning. Figures 3.2 & 3.3 show damage of the most susceptible first row of OHCs. Figure 3.4 shows more extensive OHC damage extending into rows 2 & 3. In all these cases, the IHCs can be seen to be intact.

Initially, in this experimental study, it was hoped to produce cochleas which, as well as total OHC loss in the basal region, also had normal apical areas with all haircells intact. This pattern of degeneration was desirable because the recording of normal responses in fibres from such regions would act as a control for the possible occurrence of acute cochlear pathology. For example cochlear hypoxia caused by low systemic blood pressure or an impaired cochlear blood supply would produce a deterioration in the threshold and tuning properties of cochlear fibre responses (e.g EVANS, 1972) which could be confused with any abnormal responses associated specifically with the loss of hair cells. Such patterns of degeneration were produced e.g. as shown in figure 3.1a, cochleograms E and F.

Most kanamycin treated cochleas did not have extensive areas of intact OHCs in all three rows because of the vulnerability of row 1 OHCs. Fortunately, the need for this as a control for the possibility of acute deterioration became less critical because:

[1] There was usually a close similarity between the patterns of degeneration in left and right cochleas.



Figures 3.1a & b (see over for 3.1b). Cochleograms showing the pattern of hair cell degeneration caused by kanamycin poisoning. All the cochleograms shown are from the left cochleas of 20 GPs given kanamycin, 400 mg/kg/day s.c. for 8-10 consecutive days. The animals were sacrificed 2-10 weeks after treatment.

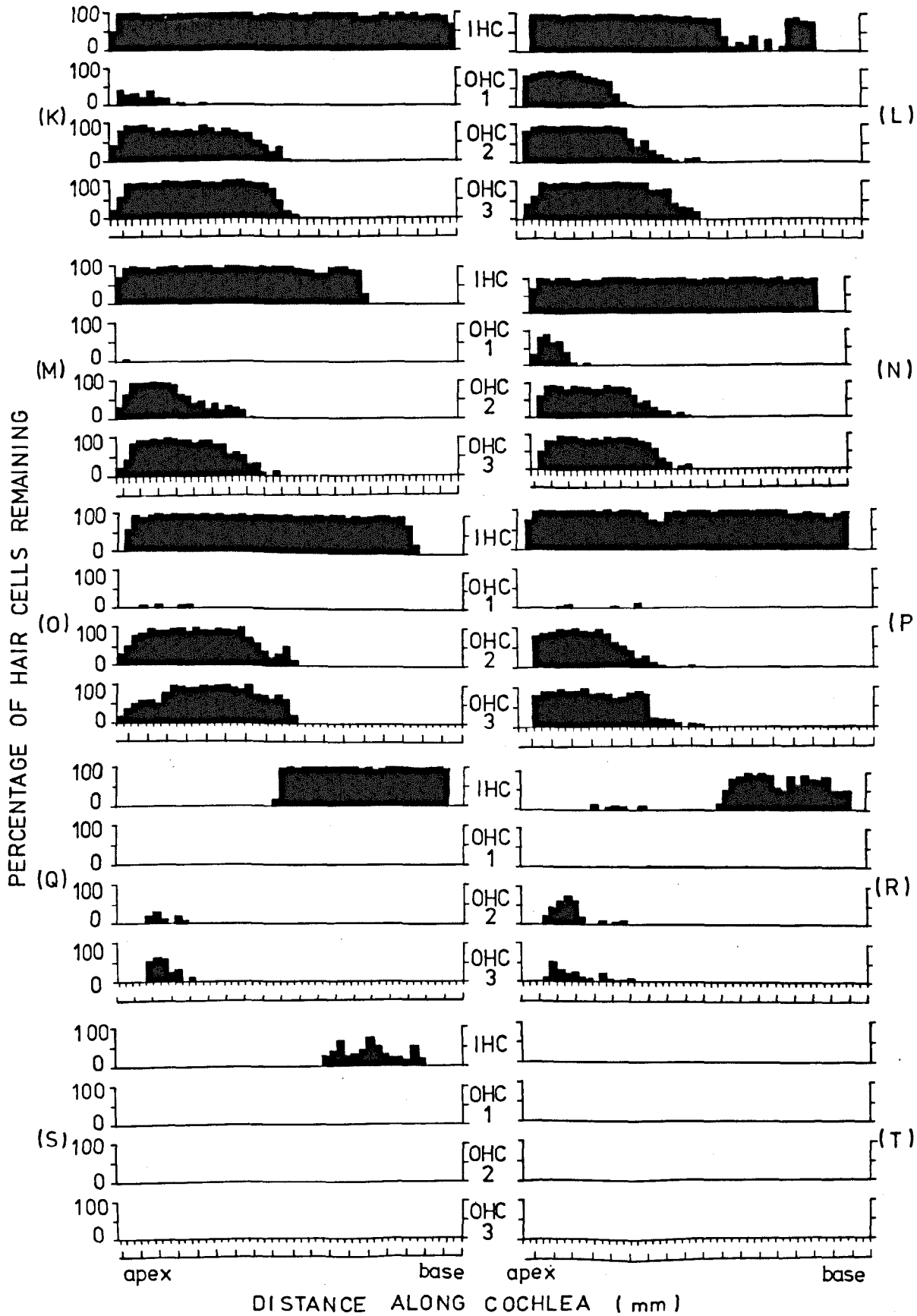


Figure 3.1b see figure 3.1a for legend.

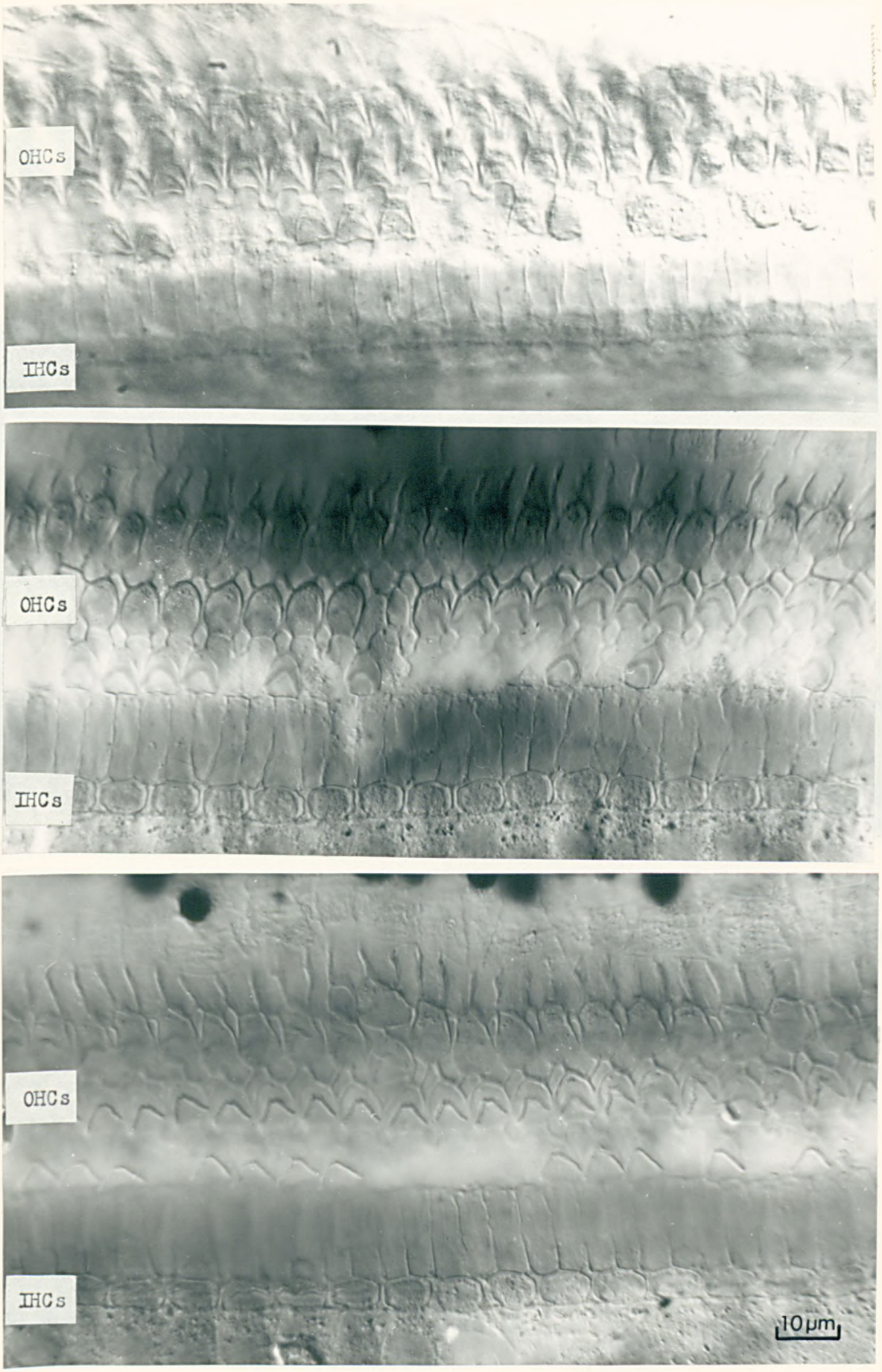


Figure 3.2 Photomicrographs of surface preparations from regions of GP organ of Corti damaged by kanamcyin. In these specimens, only the OHCs in row 1 have been affected. The other rows of OHC and the IHCs are intact. Microscope optics were Nomarski differential interference contrast.

Figure 3.3 Photomicrograph of surface preparation of a region of organ of Corti in a Kanamycin treated GP. In this specimen, most of the OHCs in row 1, and some of the OHCs in rows 2 and 3 have degenerated. The IHCs are intact. Microscope optics: Nomarski differential interference contrast.

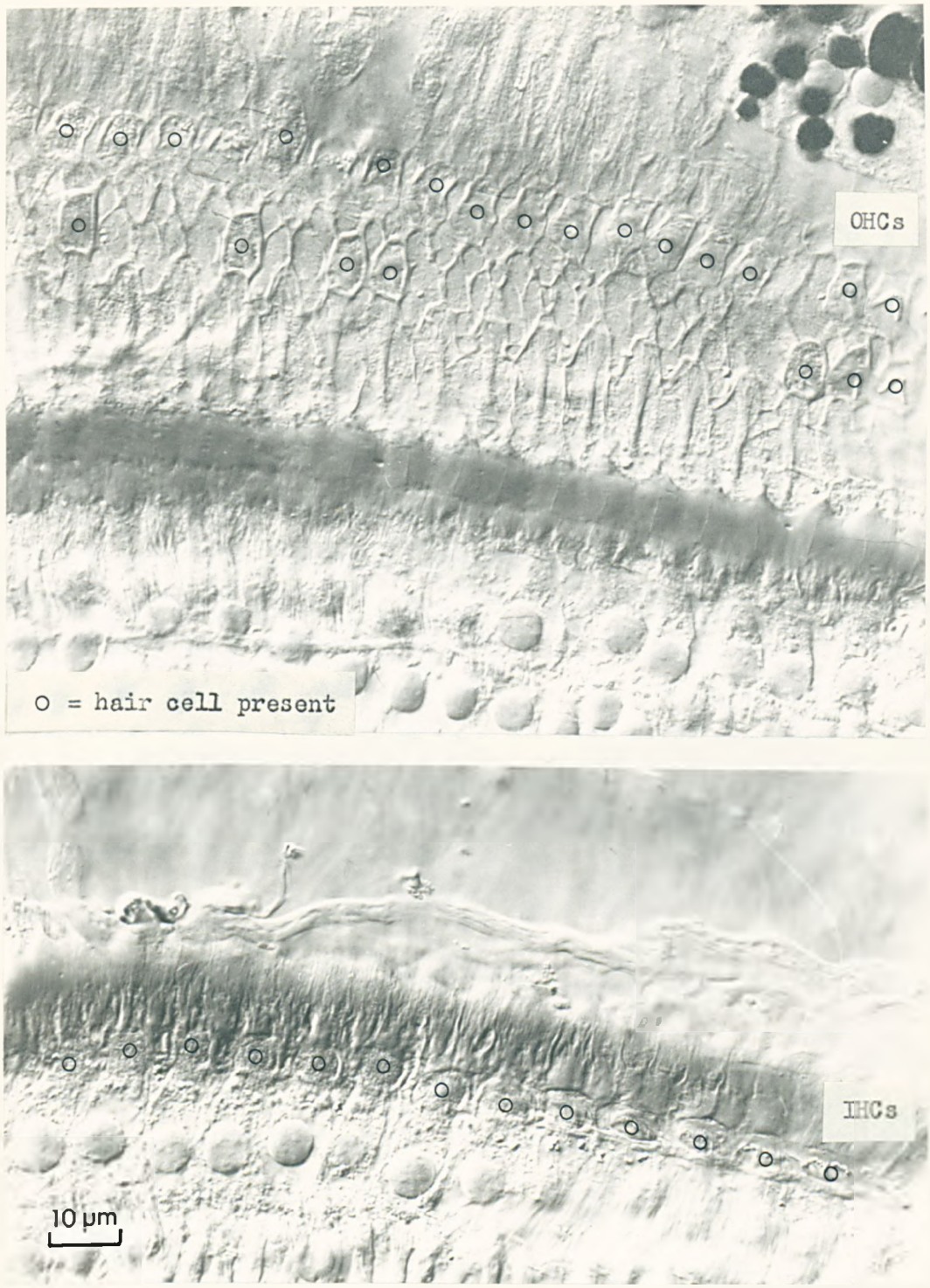


Figure 3.4 A Photomicrograph showing surface preparation of a region of GP organ of Corti damaged as a result of kanamycin poisoning. In this specimen there is total loss of row 1 OHCs and only a few OHCs remain in row 2. Most of Row 3 OHCs are intact, as are all the IHCs. In the upper photograph the IHCs are out of the plane of focus. IHCs are shown separately in the lower photograph. Microscope optics: Nomarski differential interference contrast.

a). A new anaesthetic technique (section 2.3) was developed, the use of which considerably lessened the occurrence of cochlear hypoxia caused by respiratory and cardiovascular depression.

b). It became obvious during the course of this study that the CAP audiogram reflected sufficiently well the functional state of the cochlea. Thus any change in the cochlear thresholds would be indicated by the comparison of the CAP audiogram, measured very early in the experimental proceedings (before any physiological trauma was likely to have occurred), with subsequent recordings (see section 4.1).

3.2 THE OTOTOXIC EFFECTS OF KANAMYCIN ON ALBINO GUINEA PIGS.

The kanamycin dosage regime described previously is only adequate for producing extensive haircell loss in pigmented GPs. Albino animals were much more resistant to kanamycin[2] as figures 3.5 a & b illustrate. The cochleograms from 8 GPs (from left and right ears except the case of GP 84) show relatively little degeneration of hair cells. The same dosage was used as for the pigmented animals (400 mg/kg/day) but even after administration of this dose for 14 - 16 days (GPs 69, 84, 85, 86, 87) there was relatively little cochlear damage compared with that produced in pigmented animals.

Pigmented GPs were used in the experiments described in this dissertation because of the difficulties in producing a OHC loss in the albino GP.

3.3 HAIR CELL DAMAGE AFTER LONG TERM SURVIVAL FROM KANAMYCIN TREATMENT.

The cochleograms (for pigmented GPs) illustrated in figures 3.1 a & b were of cochleas 2-8 weeks after kanamycin was administered. Figure 3.6 shows cochleograms of 5 cochleas more than 10 weeks after kanamycin administration.

These results show that in the long term, not only do the OHCs in the basal regions of the cochlea degenerate, but so also do the IHCs. In all cases, the IHCs loss occurred only in regions where there was total OHC loss; if any OHCs remained, the IHCs did not degenerate in the long term. This is illustrated in the top left hand cochleogram of figure 3.6, where very few OHCs are present along a 2 - 3 mm mid cochlear region, but the IHCs in that region remain intact.

The findings reported in this results chapter are discussed in chapter 6.

---

2 see page 112.

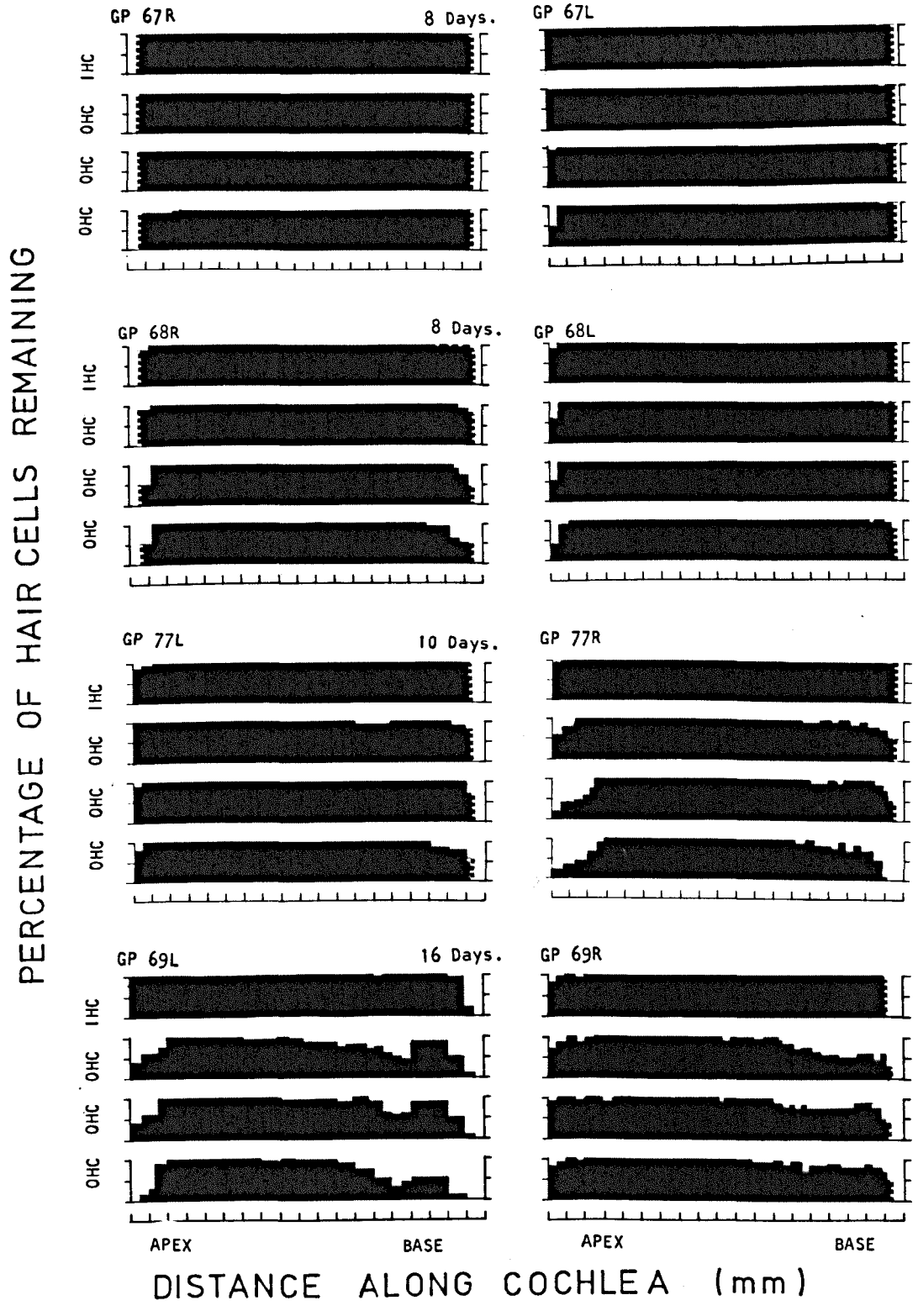


Figure 3.5a & b (see over for 3.5b). The ototoxic effects of kanamycin in albino GPs. Cochleograms of eight albino GPs (left and right cochleas except GP 84) showing the hair cell loss caused by kanamycin (400 mg/kg S.C.) given daily for the number of days indicated. The cochleas were investigated 2-6 weeks after final kanamycin injection.

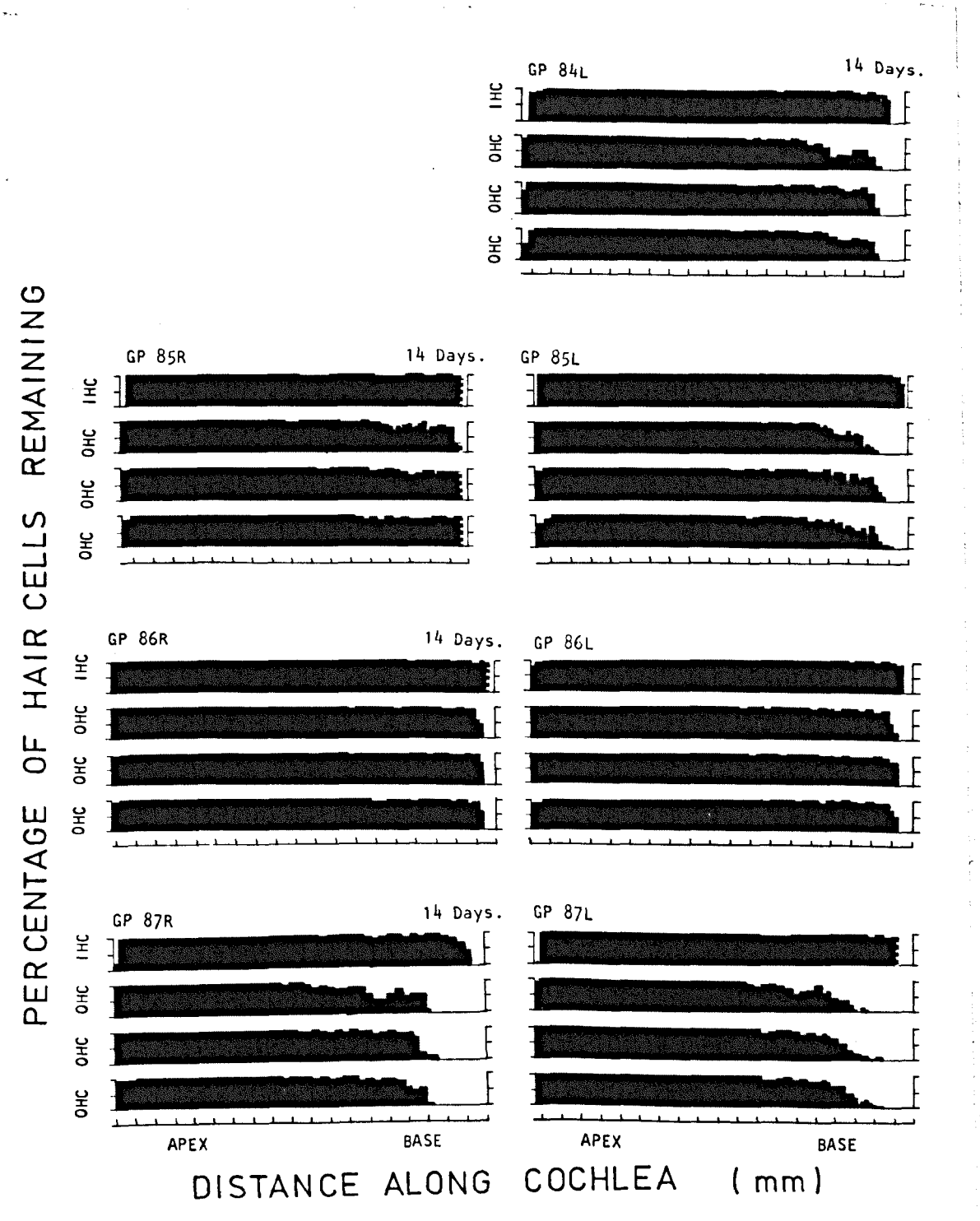


Figure 3.5b see 3.5a for legend.

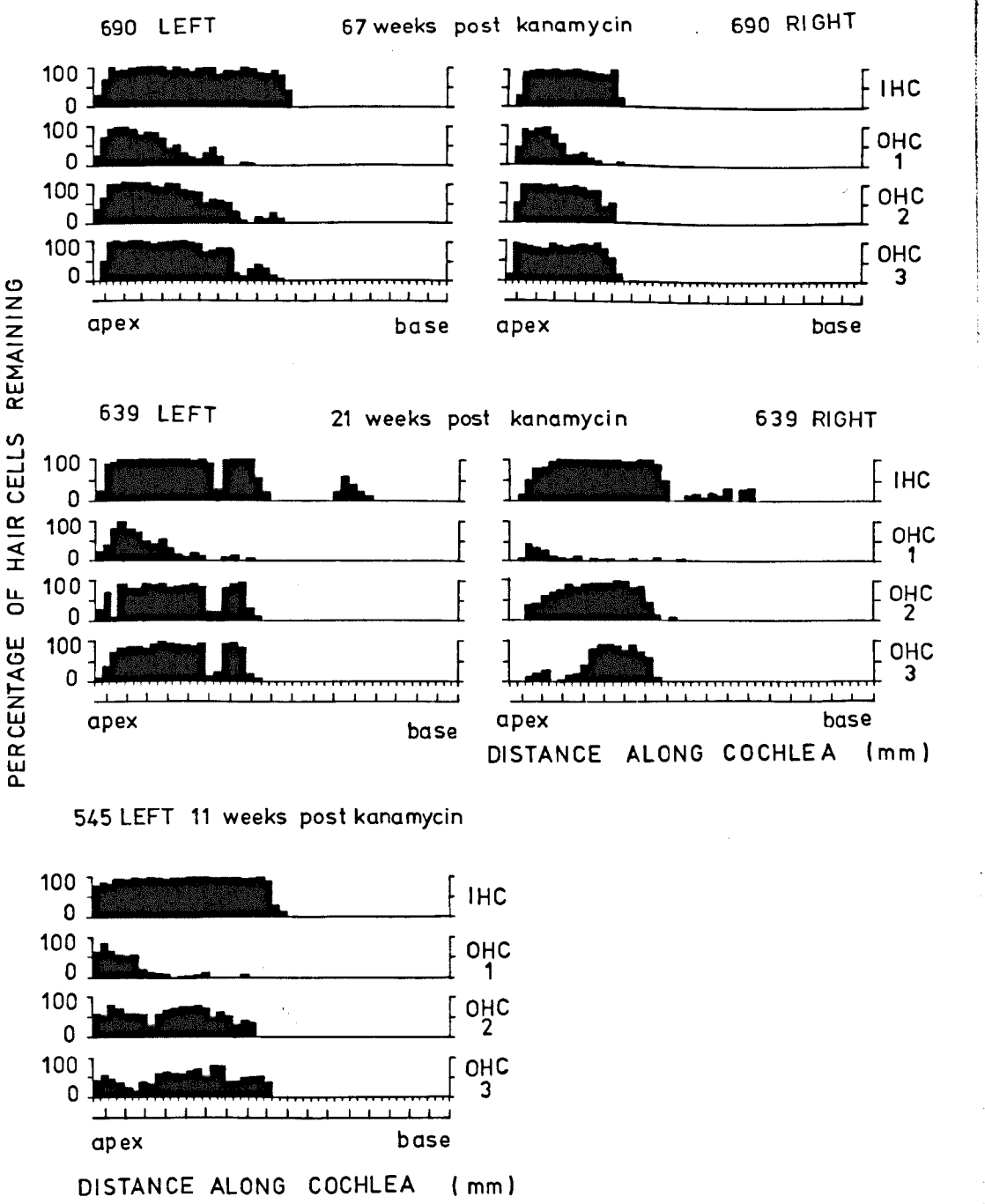


Figure 3.6 Long term inner and outer hair cell degeneration in kanamycin treated GPs. Cochleograms of three pigmented GPs (left and right cochleas except 545). The animals were sacrificed 67 weeks, 21 weeks and 11 weeks after kanamycin administration (400 mg / kg / day s.c. 8 - 10 days).

CHAPTER 4.

RESULTS OF COCHLEAR FIBRE STUDIES.

4.1 GENERAL.

4.2a SPONTANEOUS DISCHARGE RATES IN NORMAL AND PATHOLOGICAL COCHLEAR FIBRES.

4.2b THE RELATIONSHIP OF SPONTANEOUS ACTIVITY TO THE MINIMUM THRESHOLDS AND TUNING PROPERTIES COCHLEAR FIBRES.

4.3a THE MINIMUM THRESHOLDS OF NORMAL COCHLEAR FIBRES.

4.3b THE EFFECTS OF OHC LOSS ON THE MINIMUM THRESHOLDS OF COCHLEAR FIBRES.

4.4a THE EFFECTS OF OHC LOSS ON THE FTCs OF COCHLEAR FIBRES.

4.4b CHANGES IN FTC BANDWIDTH AFTER OHC LOSS.

4.4c THE DEPENDENCE OF FTC BANDWIDTH AND CUT-OFF SLOPES ON THE ELEVATION OF MINIMUM THRESHOLD.

4.1 GENERAL.

Recordings were made from over 776 cochlear fibres in 34 kanamycin treated GPs and 12 untreated control animals. The characteristic frequencies (CFs) of fibres encountered in each electrode penetration (as detailed in methods, section 2.4) were consistent with a spiral, tonotopic arrangement of fibres in the nerve. As figure 4.1 shows, fibres with high CF were encountered near the surface of the nerve, and then systematically lower CF fibres as the electrode was moved further through the nerve (obliquely across its diameter). At a depth of approximately 1.5 mm, this trend reversed, and fibres of increasing CF were encountered (see figure 4.1). Most electrode tracks were approximately 2.0mm in length. To record from fibres with CFs above 15 kHz, the electrode had to penetrate the nerve at its posterior aspect. The average number of fibres recorded per track was 15, but more than one track was usually required to obtain fibres with the wide spread of CFs desirable in these experiments.

Pulsed broad band noise was used as a search stimulus. To detect possible high threshold fibre responses, high intensities were obviously required, but judicious use was made of such stimuli to prevent threshold shifts caused by over-stimulation.

Neurones were positively identified as being first order if the spikes were positive and monophasic, and of short latency (4 ms), and if the unit was lost or regained by electrode movements of less than 10 μm. Very few units from the cochlear nucleus were encountered, and these were always anticipated from poor electrode placement e.g. in conditions of brain stem oedema, or where visibility and exposure of the nerve was poor due to uncontrollable bleeding near to the recording site.

The recording experiment was usually terminated after the second electrode track because subsequent electrode placement was often difficult (because of brain stem oedema and / or a blood accumulation around the recording site). If a deterioration in the condition of the preparation occurred (e.g. as indicated by a fall in mean systemic blood pressure, or from elevations of CAP thresholds) the experiment was terminated earlier. In any case, the cochleas were fixed immediately post-mortem for subsequent histological examination.

After plotting the cochleograms for the individual animals, and matching them to the electrophysiological data (using the cochlear frequency map described in section 2.9), the cochlear fibre responses were compared directly with the haircell degeneration in the region of the cochlea from which the

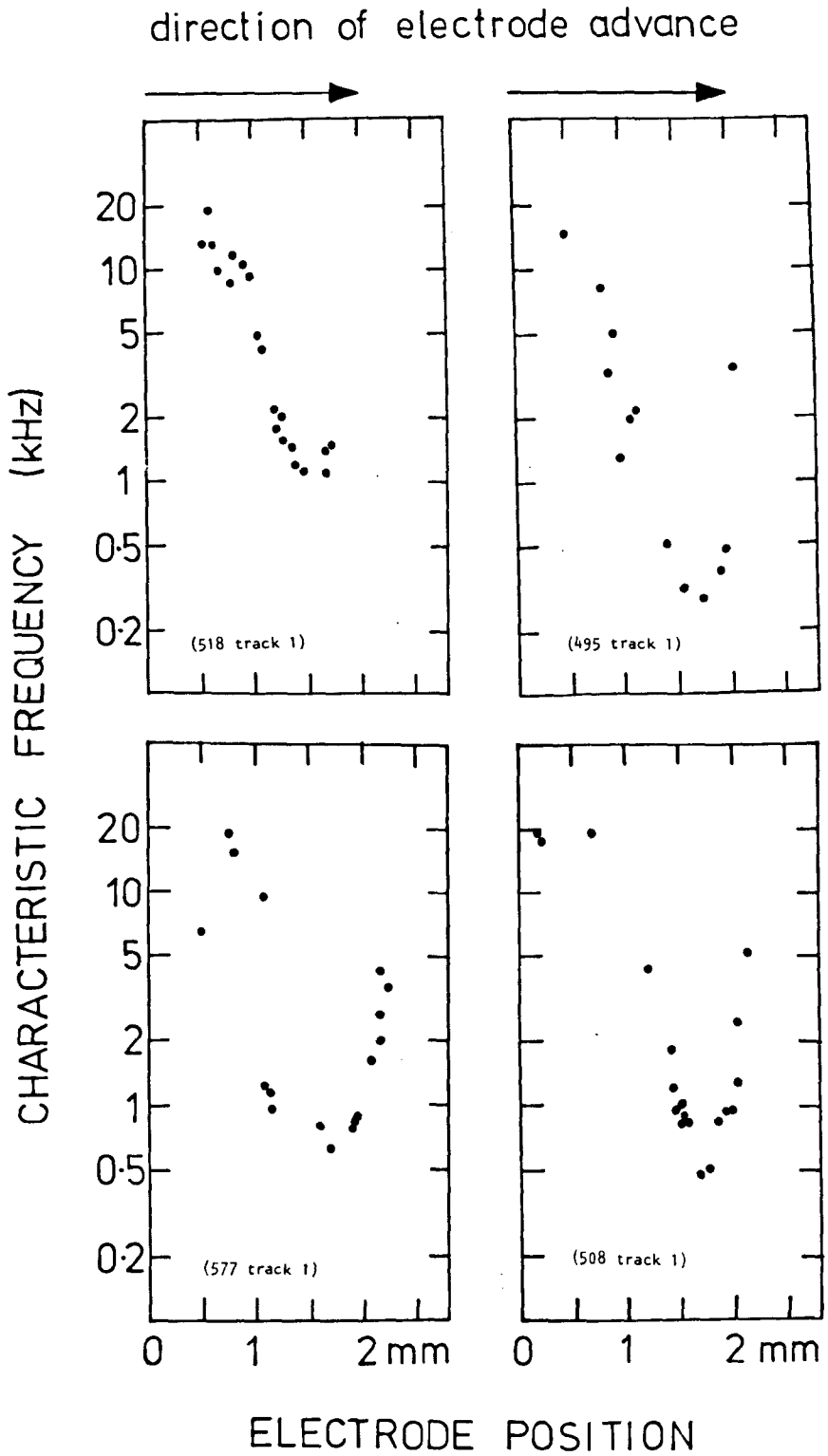


Figure 4.1 The characteristic frequency of cochlear fibre responses plotted against the position of each fibre along the electrode track in the cochlear nerve.

fibre was assumed to originate. Because some of the experimental results are presented as pooled data, the fibres were categorized according to the degree of hair cell loss in the region of cochleas from which they originate. These categories are as follows:

1). Fibres which originated in cochlear areas of total OHC loss, where the IHCs remained intact.

2). Fibres from cochlear areas with partial OHC loss (IHCs intact). In most cases, these fibres were from regions with only rows 2 & 3 OHCs remaining.

3). Fibres from areas of the cochlea, in kanamycin treated animals, with no damage and completely normal histological appearance.

4). Fibres from normal control GPs.

5). Categorized separately were a number of fibres, from both control and kanamycin treated GPs, whose responses were abnormal because the history of the experimental proceedings indicated that the cochlea may have been hypoxic e.g. through a temporary apnoea, a period of low arterial blood pressure ($<$50 mm Hg) or a restriction of the blood supply to the cochlea caused by occlusion of the internal auditory artery. The latter was indicated by abrupt changes in the threshold of the N_1 response to click stimulation.

In the following results sections, the symbols used in the pooled data allow the above categories to be distinguished.

Before presenting the results it is appropriate to illustrate how the CAP audiogram (see section 5.1) was used to resolve any possible confusion between the effects of chronic hair cell degeneration, and acute cochlear hypoxia which could occur during the experimental proceedings. (Only if there were no such changes could abnormal fibre responses be positively associated with chronic hair cell damage.)

The middle diagram of figure 4.2 shows that cochlear fibres with CFs above 10 kHz had abnormal FTCs. However, the cochleogram below indicates that the pathology was not due to the loss of haircells; the cochlea had, in fact, a nearly normal complement of hair cells. The corresponding CAP audiograms in the top diagram indicate clearly that the functional state of the cochlea had deteriorated during the experimental proceedings. The dashed curve shows the initial CAP audiogram, the dotted and continuous curves show respectively, the state of the cochlea immediately before and after the single fibre recordings were made, and reflect quite accurately the elevation in the minimum thresholds of the high CF fibres.

The arrows to the right of the CAP audiogram indicate the threshold (peak equivalent[1] dB SPL) of the CAP to click stimuli, ascertained at the time

[1] Peak equivalent of pure tone at 10 kHz.

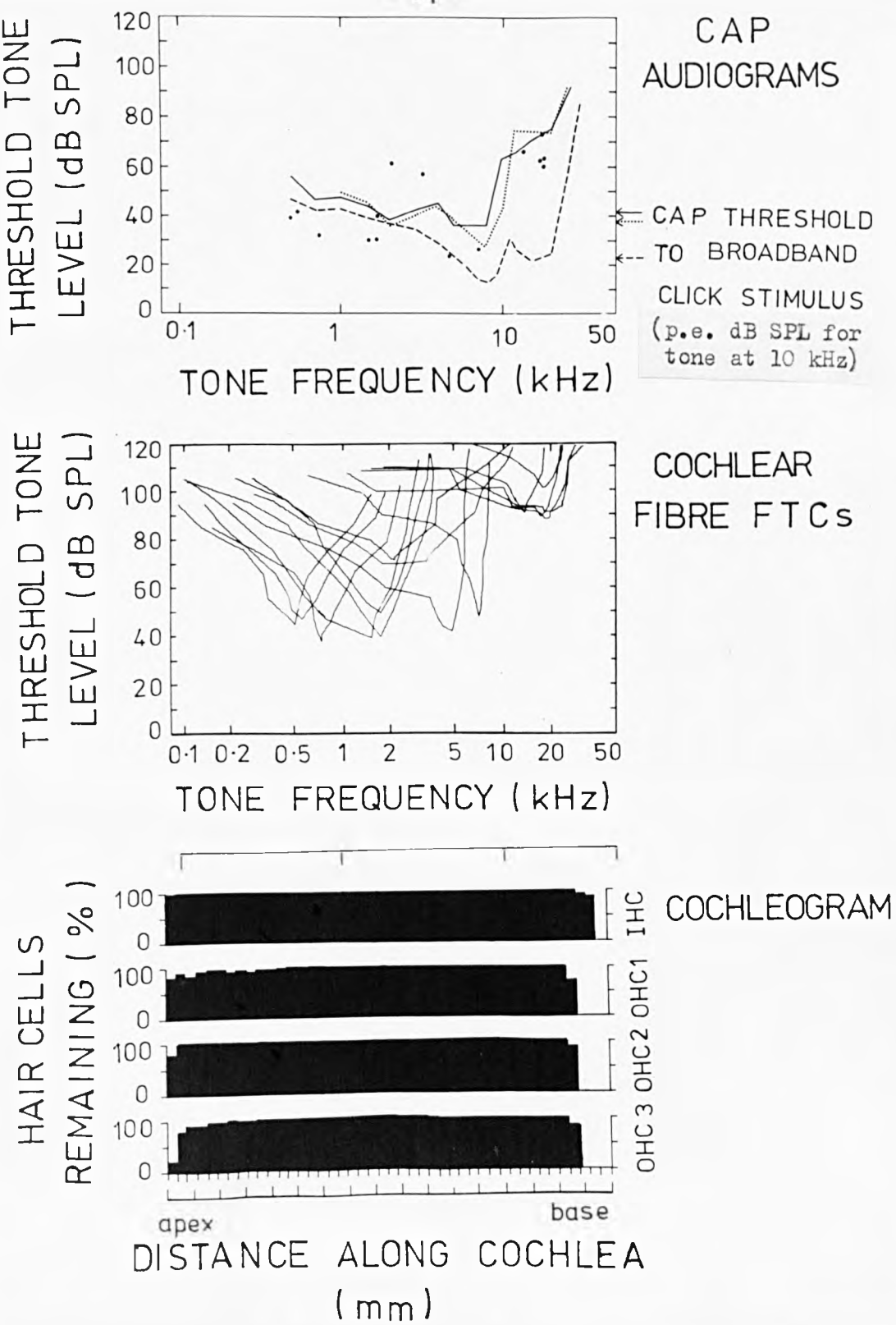


Figure 4.2 The use of the CAP audiogram to resolve a possible confusion between the effects of chronic hair cell degeneration, caused by kanamycin poisoning, and acute cochlear hypoxia which could occur during the experimental proceedings. The top diagram shows GP CAP audiograms measured before (dashed curve) and after electrode placement (dotted & continuous curves). The arrows to the right indicate the CAP threshold to a broadband click stimulus (in peak equivalent dB SPL). The middle diagram shows the cochlear fibre FTCs and the lower diagram is the cochleogram showing the state of degeneration of hair cells at the time of the electrophysiological recording. The change in the CAP audiogram during electrode placement indicates clearly that the cochlear fibre FTCs with CFs above 10 kHz are abnormal because of an acute cochlear insult rather than because of chronic hair cell damage.

of the corresponding CAP audiograms. The 15-20 dB elevation of this click threshold (dashed arrow to dotted and continuous arrows) does not fully reflect the 50 dB elevation of the minimum thresholds of high CF cochlear fibres. The 50 μs click stimulus used for CAP threshold monitoring was not optimal for reflecting activity of high CF fibres (because of the energy minimum at 20 kHz). However, any stimulus with a flat energy spectrum will be inadequate for indicating threshold shifts at high frequencies, e.g.> 10kHz, if such threshold shifts occur independently of changes at lower frequencies.

4.2a SPONTANEOUS DISCHARGE RATES IN NORMAL AND PATHOLOGICAL COCHLEAR FIBRES.

The mean spontaneous discharge rate was measured for 310 cochlear fibres (over at least 20s of sample time). If no firing occurred during the sample period, the fibre was designated as having zero spontaneous activity.

Figure 4.3 shows the distribution of spontaneous discharge rates, in spikes/s, plotted against the CF of the fibres (divided into the categories described previously; section 4.1). The distribution of spontaneous activity in these categories is shown in the histograms of figure 4.3. For fibres from normal cochlear areas[2] (histogram A) 89% had spontaneous discharge rates greater than 5/s. The majority of spontaneous discharge rates (69%) were between 20-80 spikes/s.

In contrast to fibres from normal cochlear areas, a high proportion of fibres from areas of OHC degeneration had low or zero spontaneous rates of discharge. Almost half the fibres from areas of total OHC loss were silent unless stimulated. The range of spontaneous rates in histogram B was similar to that of normal fibres (histogram A) although the distribution was different.

Many fibres (30%) from areas of partial OHC loss (mainly where only 2 rows of OHC remained) had zero or near zero spontaneous discharge. The rate of discharge of the other fibres in this category were predominantly between 20-50 spikes/s (histogram C).

In fibres from control GPs, but which had suffered physiological insult during the course of the recording experiment, 30% had low or zero spontaneous rates of activity (histogram D).

4.2b THE RELATIONSHIP OF SPONTANEOUS ACTIVITY TO THE MINIMUM THRESHOLDS AND TUNING OF COCHLEAR FIBRES.

Figure 4.4 shows the rate of spontaneous discharge of normal and pathological fibres plotted against the minimum thresholds of the fibres (in dB

2 Responses of fibres recorded from kanamycin treated GPs, but which came from regions of intact inner and outer hair cells, did not differ (in respect of all the properties investigated in the present study) from the responses of normal fibres from untreated animals. These two categories of fibres are therefore pooled in histogram A.

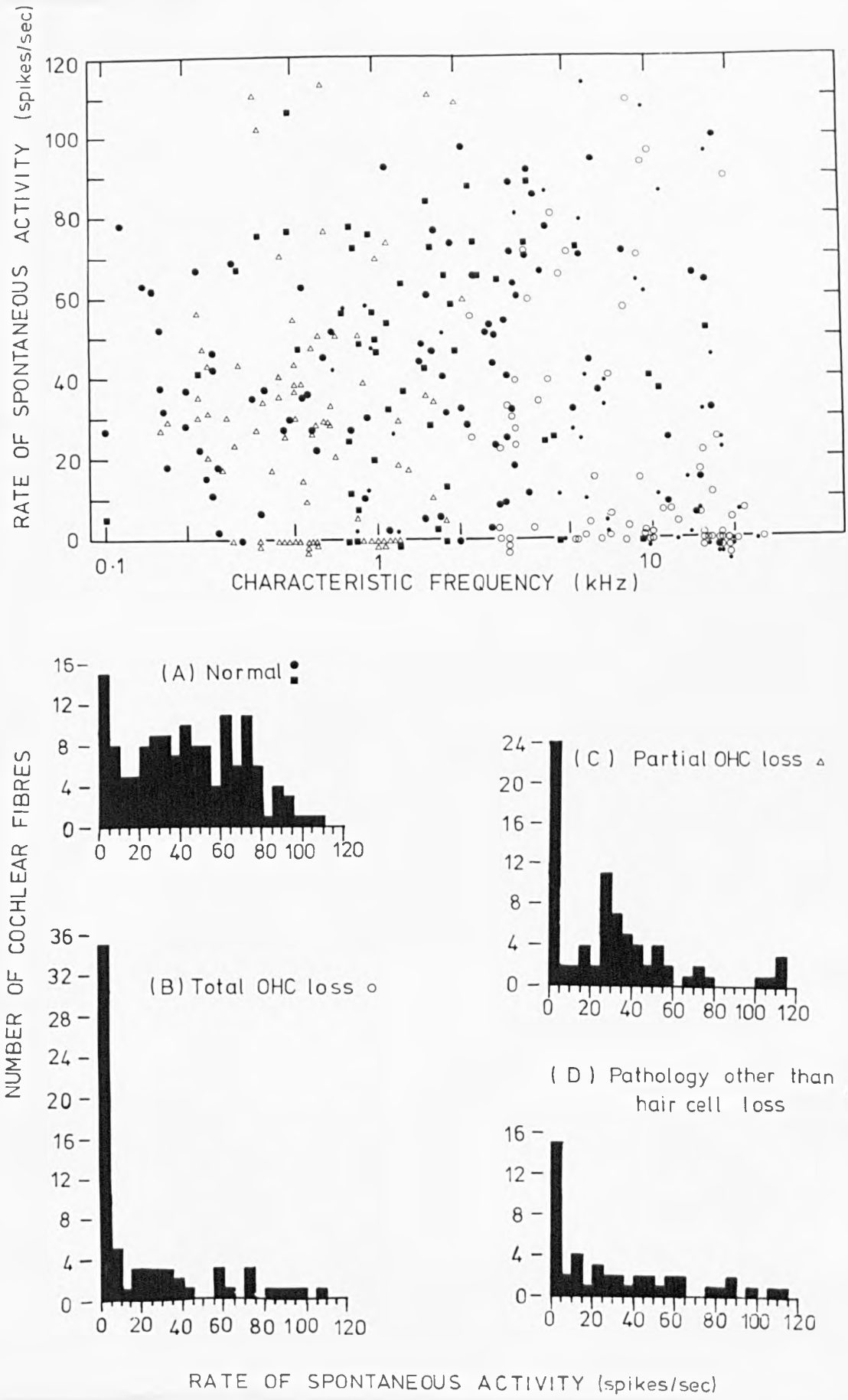


Figure 4.3 The rates of spontaneous activity of cochlear fibres from normal and kanamycin treated GP cochleas, plotted against the CF of each fibre. The histograms show the distribution of the rates of spontaneous activity for the separate fibre categories indicated.

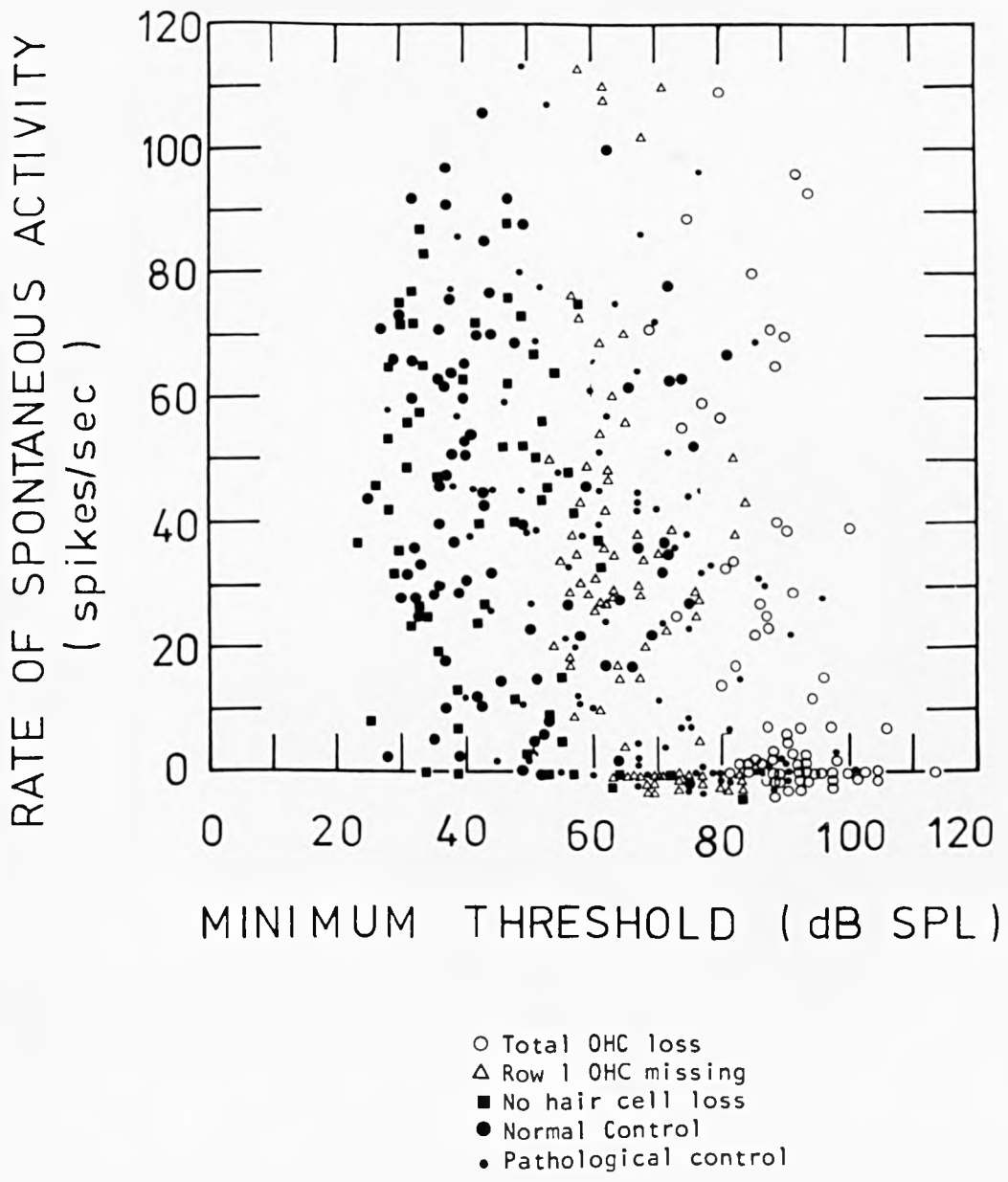


Figure 4.4 The rate of spontaneous activity of cochlear fibres from normal and kanamycin treated GPs, plotted against the minimum threshold of each fibre.

SPL). The pathological fibres (open symbols and small filled circles) had elevated minimum thresholds, and many of them also had low rates of spontaneous activity. In this respect there was a relationship between minimum threshold and spontaneous activity. However, this relationship only applies to pathological cochleas. For normal cochlear fibres there was no such obvious relationship, as figure 4.5 shows. In this figure, the spontaneous activity of fibres from normal cochlear areas is plotted against the minimum thresholds. The low correlation coefficient indicates that there was no significant relationship between minimum threshold and spontaneous activity.

The tuning of normal cochlear fibres was also not obviously related to their rates of spontaneous activity. Figure 4.6 shows the spontaneous activity plotted against the 10 dB bandwidth of the fibre FTCs. The bandwidth values have been taken from fibres of all CFs, and to normalize these values they were measured on a square root frequency scale (EVANS, 1972 after ROSS, H.F). The correlation coefficient of the data in figure 4.6 is close to zero (-0.181) and taken with the obvious scatter of data points indicates no strong relationship.

Discussion of thesedata from pathological and normal cochlear fibres is in section 7.1.

4.3a THE MINIMUM THRESHOLDS OF NORMAL COCHLEAR FIBRES.

Figure 4.7 shows the minimum thresholds of over 500 cochlear fibres (from 28 kanamycin treated GPs and 12 control GPs) plotted against their CFs. The filled circles are minimum thresholds of cochlear fibres from normal control GPs, and the filled squares are those of fibres from unaffected cochlear areas of kanamycin treated GPs. (These are categories 3 and 4, as defined in section 4.1). These two categories of fibres overlap completely with regard to their minimum threshold properties (as with all other properties) and it can be inferred that at the time of the electrophysiological recording, cochlear areas with a normal hair cell complement gave rise to normal cochlear fibre responses despite having presumably been exposed to kanamycin weeks earlier. That is, there was no functional abnormality in cochlear areas which have resisted damage. In these normal data, the minimum thresholds approach to within 10-15 dB of the behavioural threshold (dashed curve of figure 4.7 - from HEFFNER et al. (1971) corrected for the frequency response of the external meatus and pinna by EVANS). For frequencies above 15 kHz there is a discrepancy between the behavioural audiogram and the minimum thresholds of normal cochlear fibres. This is discussed fully in section 7.2.

It can be seen from figure 4.7 that the spread of normal minimum thresholds

fig.4.5

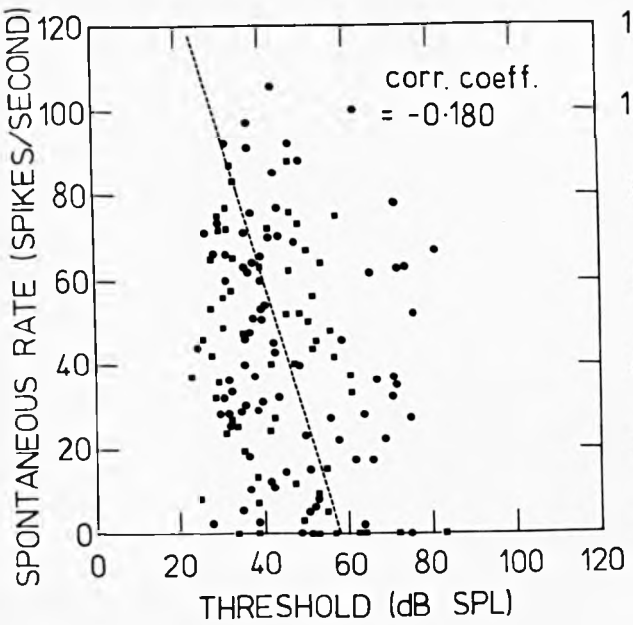


fig.4.6

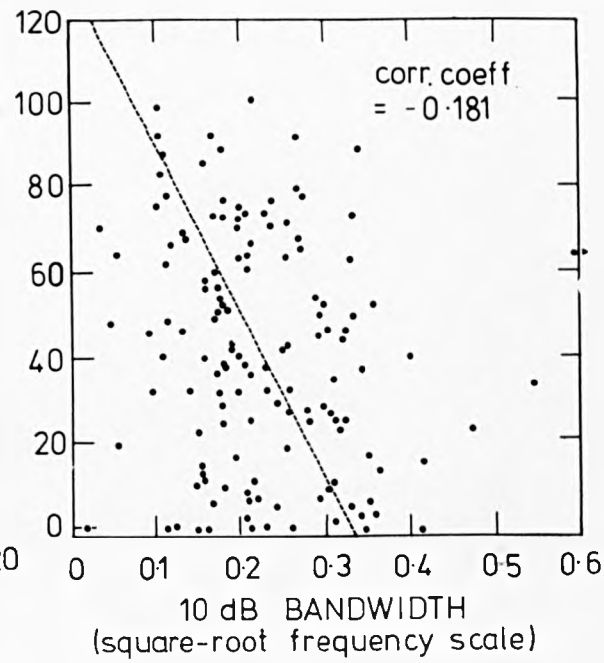


Figure 4.5 The rate of spontaneous activity of normal cochlear fibres from GP plotted against the minimum threshold of each fibre. The linear regression of the thresholds on to the spontaneous rates of discharge is shown by the dashed line.

Figure 4.6 The rate of spontaneous activity of normal cochlear fibres from GP plotted against the 10 dB bandwidth of the FTC of each fibre. The 10 dB bandwidth is measured on a square root frequency scale to normalize tuning values across the frequency range over which the data was pooled (EVANS, 1972 after suggestion of H.F. ROSS). The linear regression of bandwidth on to spontaneous activity is shown by the dashed line.

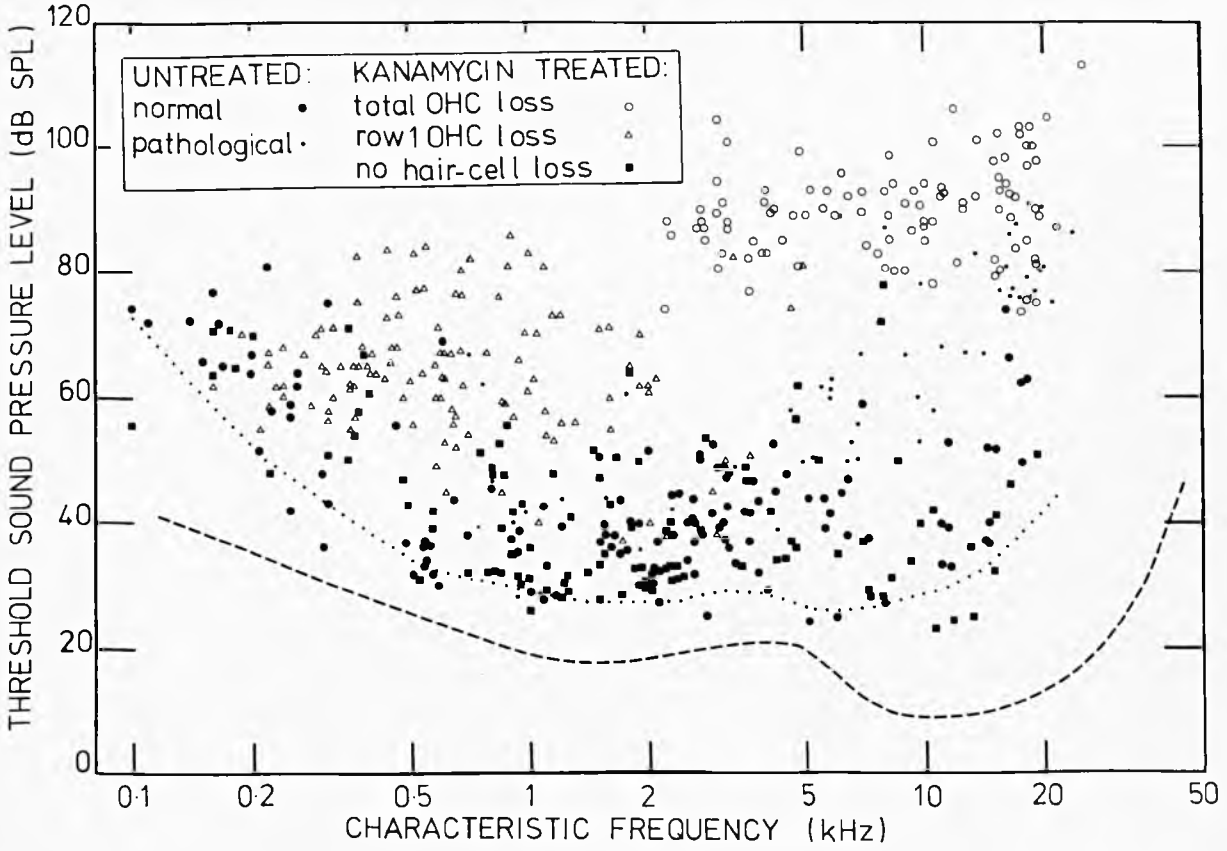


Figure 4.7 The minimum thresholds of over 500 cochlear fibres from 12 normal and 28 kanamycin treated GPs, plotted against the CF of each fibre. The fibres are categorized as indicated. The dashed curve represents the behavioural audiogram for the GP (HEFFNER et al. 1971, corrected for the frequency response of the external meatus and pinna by EVANS). The dotted curve, drawn near to the lowest minimum thresholds, is the reference against which the minimum threshold elevations referred to in the text were measured.

at any one frequency is about 40 dB. In individual animals the spread is less than 30 dB. This can be observed in figure 4.9 which shows unselected FTCs from two control animals.

4.3b THE EFFECTS OF OHC LOSS ON THE MINIMUM THRESHOLDS OF COCHLEAR FIBRES.

As shown in figure 4.7, the minimum thresholds of fibres from regions of total OHC loss (IHCs intact; open circles) were all in excess of 70 dB SPL, most of them were between 75-100 dB SPL. In no instance did cochlear fibres from regions of total OHC loss have normal minimum thresholds, all were elevated 40-60 dB above normal values. Fibres from regions of partial OHC loss (open triangles) had threshold elevations which were typically 20-30 dB.

Figures 4.10-4.21 show the FTCs from 12 kanamycin treated GPs. Below the electrophysiological data is the cochleogram, appropriately scaled and aligned, according to the frequency map used (section 2.9), for comparison with electrophysiological data. From theseindividual animal data it can be seen that fibres from regions of partial OHC loss had a partial elevation of minimum threshold, for example in figure 4.11 between 3-5 kHz, and in figure 4.12 between 1-3 kHz. (The dotted or dashed curve in each figure indicates the approximate values of normal minimum thresholds and is derived from the dotted curve in figure 4.7.)

The relationship between the elevation in minimum threshold and the amount of OHC loss is shown in figure 4.8. The elevation in minimum threshold was measured from the hypothetical normal minimum threshold value shown by the dotted curve in figure 4.7. The percentage of OHC loss for each fibre was measured over a 1.2 mm cochlear region, centred at the position assumed to correspond to the CF of the fibre. 1.2 mm was chosen for convenience because, in the cochleogram format used for this study, it represents 3 hair cell binwidths (equivalent to a region containing 450 OHCs). Only the minimum threshold of fibres with CFs between 0.2-15 kHz are plotted in figure 4.8, thus avoiding fibres at the extremes of the GP hearing range. There is a considerable scatter of data points in figure 4.8, much of which is the inevitable result of pooling data from many animals (28 kanamycin treated GPs). Nevertheless, the data suggest a relationship between the minimum threshold of a cochlear fibre and the degree of OHC loss in the region of the cochlea from which that fibre emanates.

Returning to figure 4.7, the small points are the minimum thresholds of fibres from untreated control animals whose cochleas were in poor physiological condition due to low systemic blood pressure, or an interference with the local blood supply to the cochlea. Many fibres of this type had

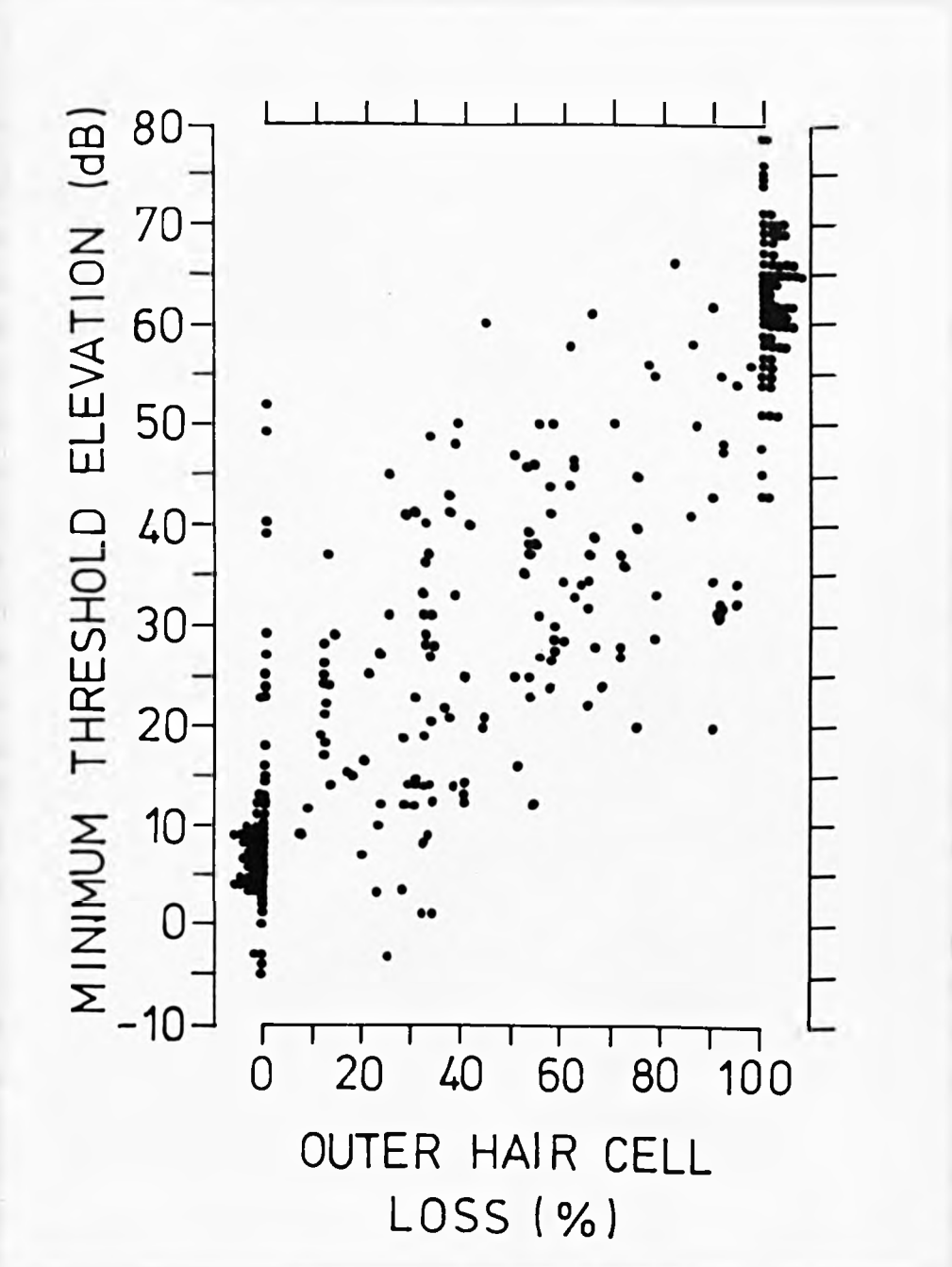


Figure 4.8 The elevation of minimum threshold of cochlear fibres* (from normal and kanamycin treated GPs) plotted against the percentage of OHC loss in the region of the cochlea (1.2mm) from which each fibre is assumed to originate. The threshold elevation is measured from a hypothetical minimum threshold derived from the most sensitive cochlear fibres found in normal control GPs. *(fibres with CFs from 0.2 - 15 kHz).

minimum thresholds which approach the minimum thresholds of the fibres from areas of total OHC loss (open circles).

These results, demonstrating the effect of OHC loss on the minimum thresholds of cochlear fibres are discussed in section 7.2.

4.4a THE EFFECTS OF OHC LOSS ON THE FTCs OF COCHLEAR FIBRES.

FTCs were determined for 699 cochlear fibres; 162 FTCs were recorded from untreated, control GPs and 537 were from kanamycin treated animals.

The data from control GPs served for comparison with data from the kanamycin treated GPs. FTCs from two control GPs are shown in figure 4.9, and are similar to those found by EVANS (1972). Fibres with CFs above 2 kHz had FTCs which can be described as having a low threshold, sharply tuned 'tip' segment, and a high threshold, broadly tuned 'tail'. For fibres of lower CF, the FTCs were more symmetrical (on a logarithmic frequency scale).

Figures 4.10-4.21 show the FTCs from 12 kanamycin treated GPs (for clarity, only a typical sample of FTCs is shown in some of these figures). In the lower half of each figure is the cochleogram (scaled and aligned appropriately with the electrophysiological data) showing the pattern of hair cell degeneration in the cochlea at the time of the FTC recording.

The FTC data of figures 4.9-4.21 were determined by the manual method described in section 2.7b. In addition, three other methods of FTC determination were used.

1). A semi-automatic threshold following procedure in which the frequency of a (gated) tone was changed slowly (approximately 0.5 octave/minute) while its intensity was, repeatedly and by manual adjustment, increased (until the tone just stimulated the fibre) and then reduced well below threshold.

2 & 3). Two methods of completely automatic FTC determination. These experiments were carried out in conjunction with E.F. EVANS; figures 4.22 & 4.23 show some of the results. Figure 4.22 illustrates a sample of FTCs determined using a threshold following paradigm in which the intensity of a pure tone stimulus was automatically adjusted to maintain a constant threshold criterion (e.g. one or two spikes difference between a period of stimulation and an identical period without stimulation) as the frequency of the stimulus was slowly changed[3] (see EVANS & WILSON, 1975 for methods). In figure 4.23 the FTCs were determined by the threshold following paradigm as well as (in five examples shown) a method in which pure tone stimuli were presented in a pseudo-random sequence to cover a large area of frequency & intensity space (5 octaves x 60 dB; see EVANS 1974b for method). In both figures (4.22 & 4.23) the place of origin of the cochlear fibres from which the FTCs were determined is indicated above the cochleogram.

---

3 FTC determinations were made with at least one upward and one downward frequency sweep (except unit 691.013 in fig.4.23) in order to control for hysteresis in the tracking paradigm.

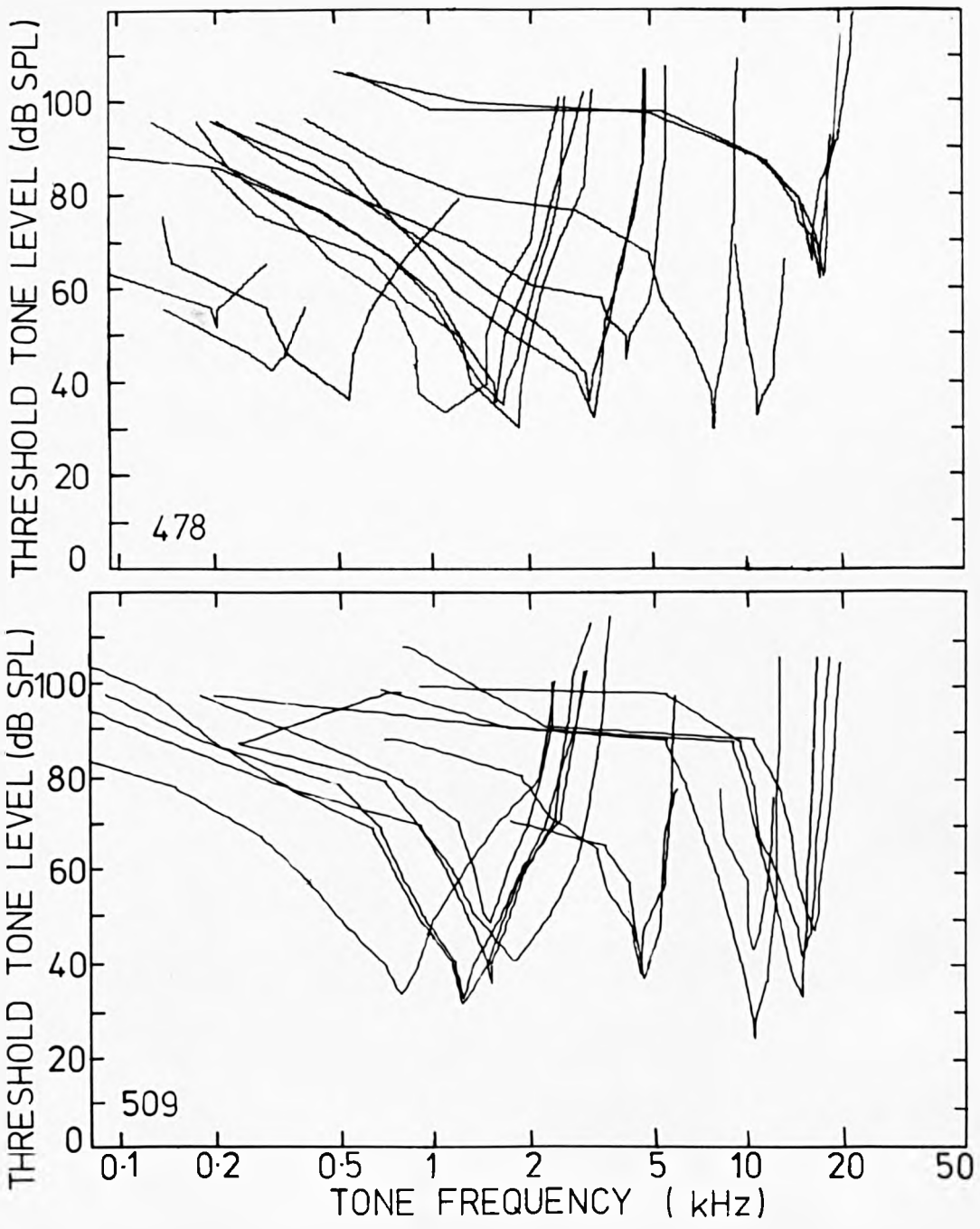
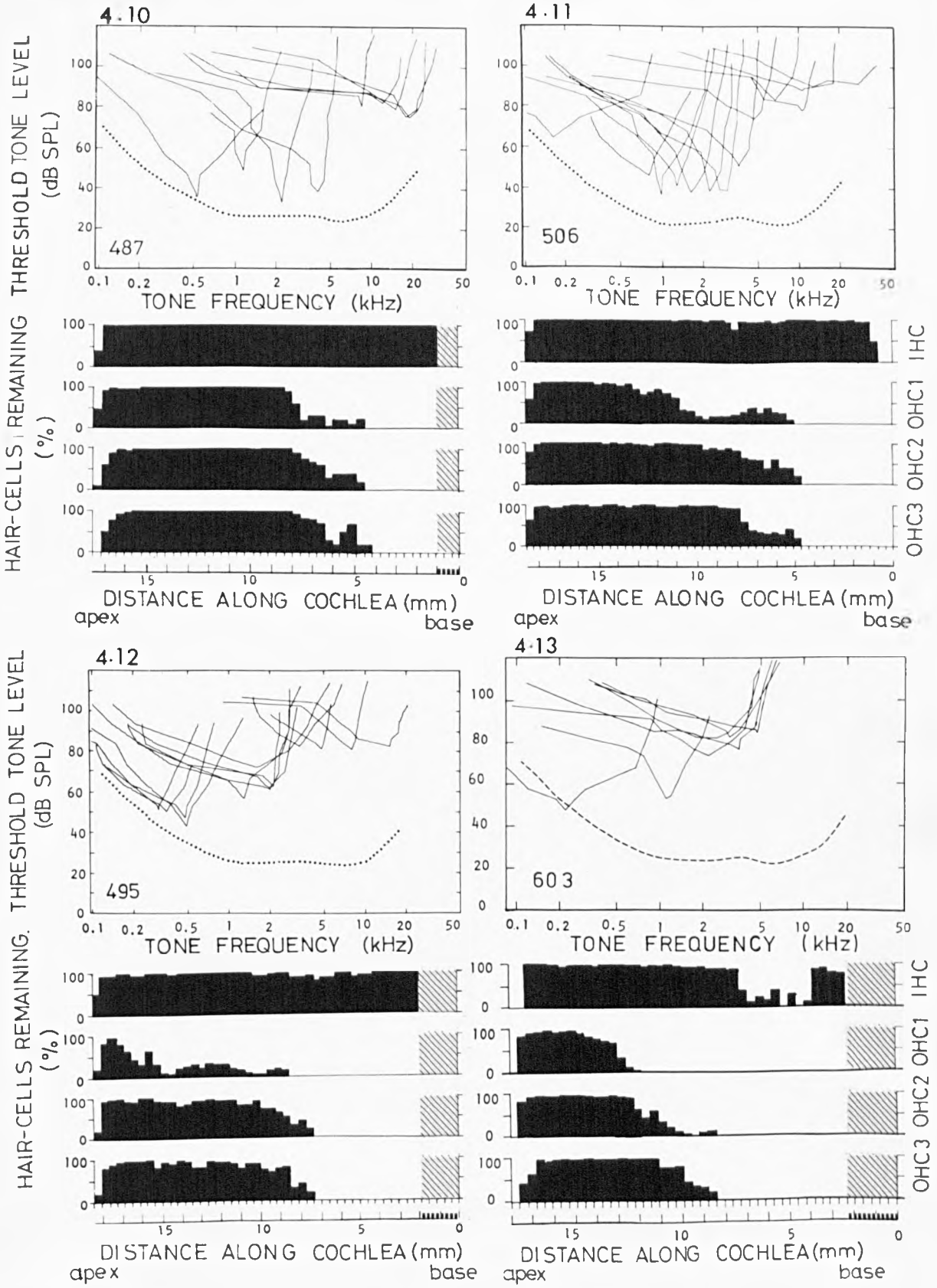
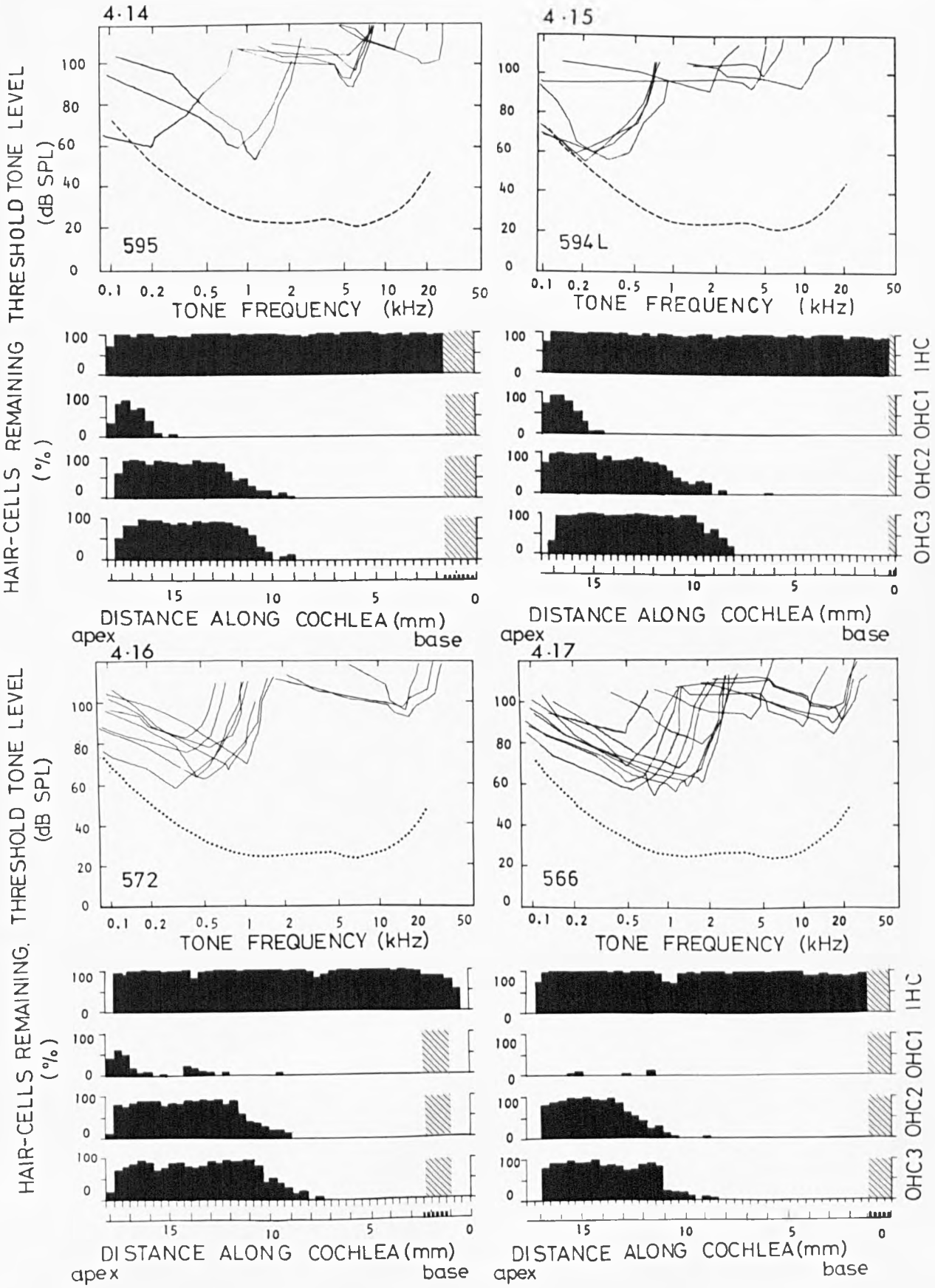


Figure 4.9 Frequency threshold curves of a representative sample of cochlear fibres from two normal GPs. Thresholds measured in dB SPL at the tympanic membrane.

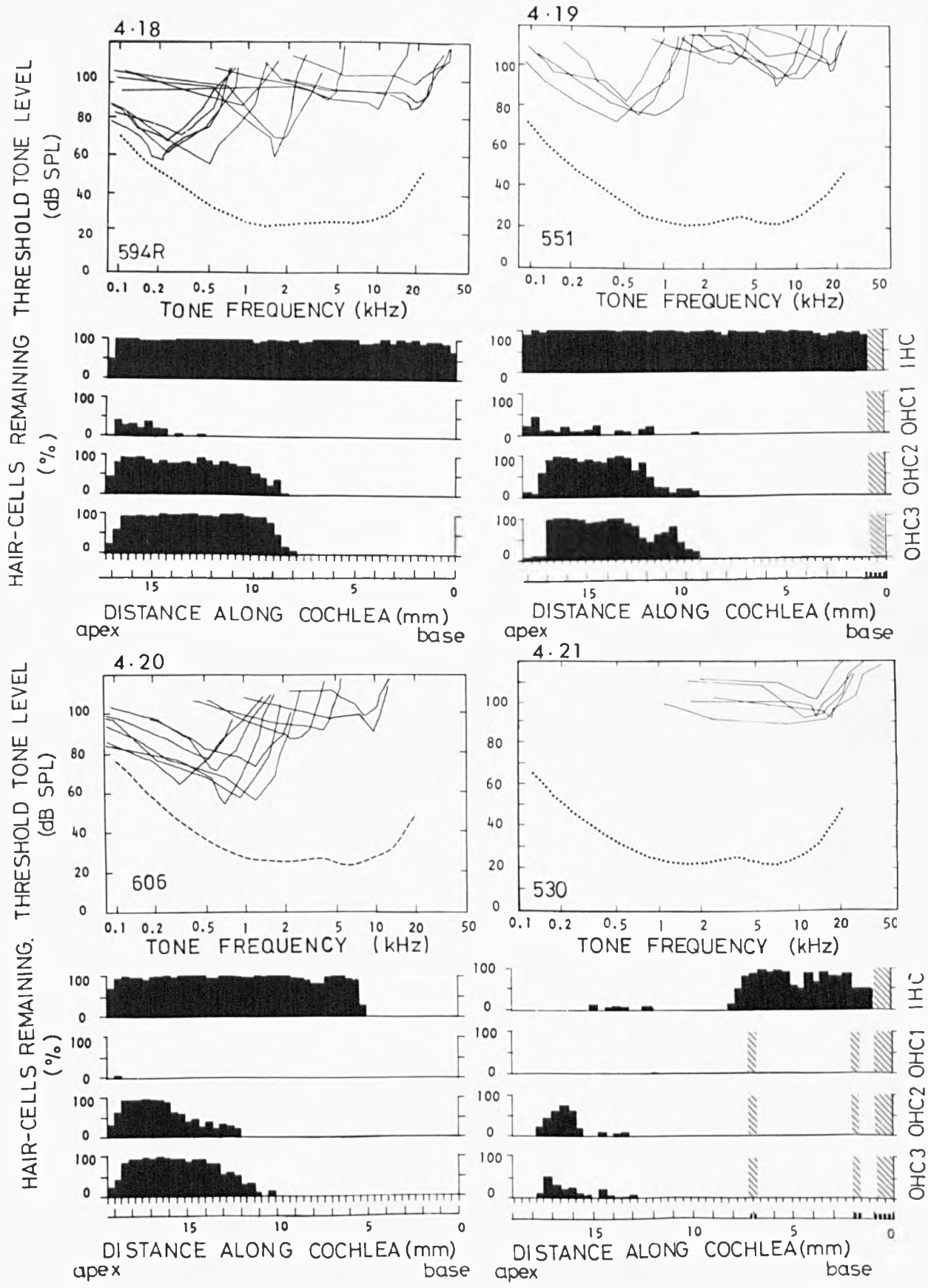


Figures 4.10-4.21 The effects of kanamycin induced hair cell loss on cochlear fibre FTCs. The top half of each figure shows a representative sample of FTCs from an individual kanamycin treated GP. The dashed or dotted curve in each case indicates the approximate minimum threshold values for normal cochlear fibres, derived from the most sensitive minimum thresholds of normal cochlear fibres. The lower half of each figure is the cochleogram showing the percentage of hair cells remaining in the cochlea at the time of the physiological recording. The striped region along the length scale of some of the cochleograms indicates areas where the histological assessment of cochlear damage was not possible.

(see over for 4.14-4.31).



Figures 4.14-4.17 see figure 4.10 for legend.



Figures 4.18–4.21 see figure 4.10 for legend.

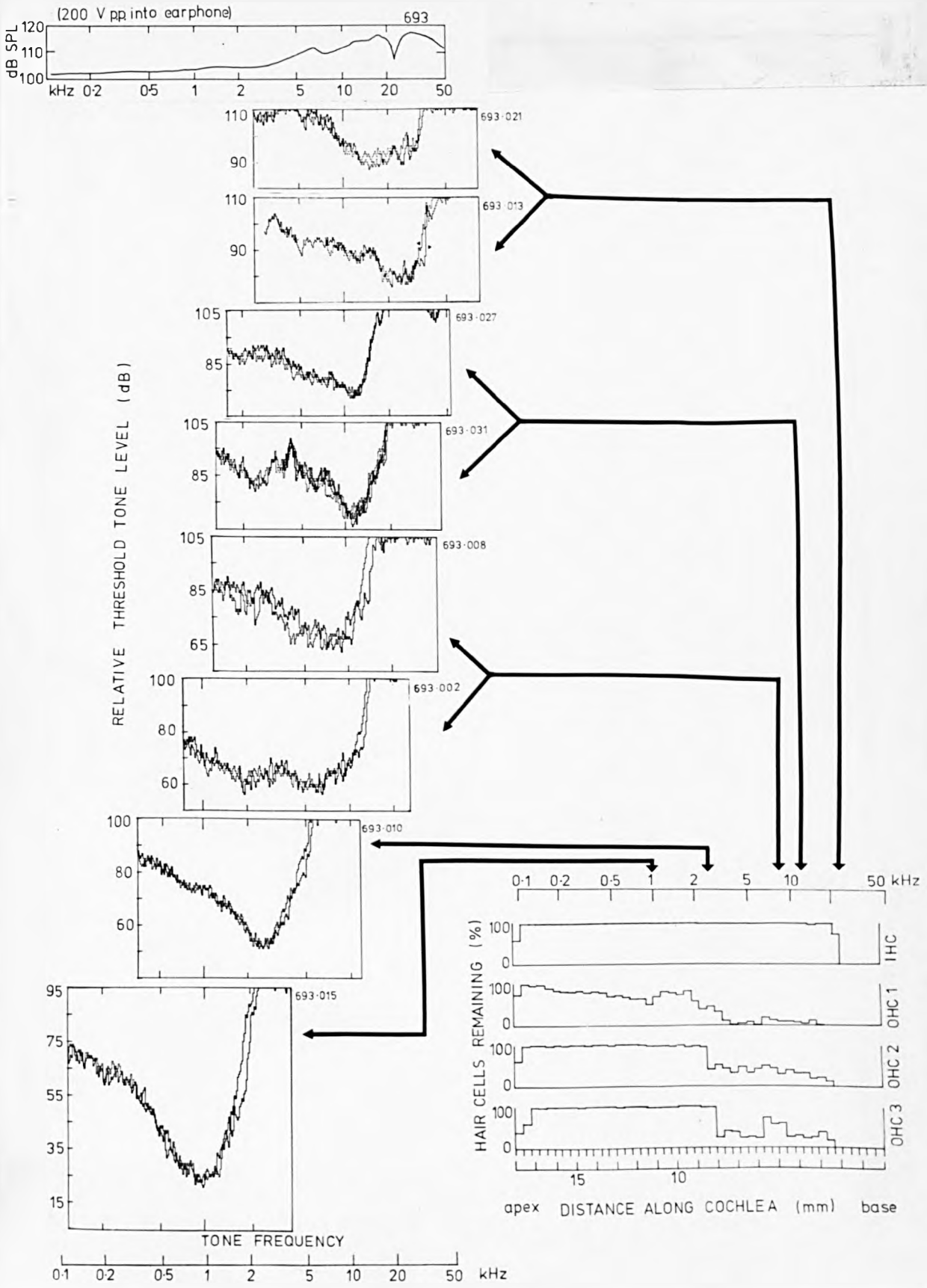


Figure 4.22 The effects of kanamycin induced hair cell loss on cochlear fibre FTCs. A representative sample of 8 FTCs from a kanamycin treated GP are shown. The FTCs were determined by an automatic threshold following paradigm (see text for details). The stimulus levels are given in relative terms of the electrical input to the condenser earphone, and they represent approximate (± 6) dB SPL at the tympanic membrane. The variation in stimulus level across frequency is indicated by the plot of dB SPL, at the tympanic membrane, for a 200 V p.p. signal into the condenser earphone. The cochleogram shows the percentage of hair cells remaining in the cochlea at the time of the physiological recording.

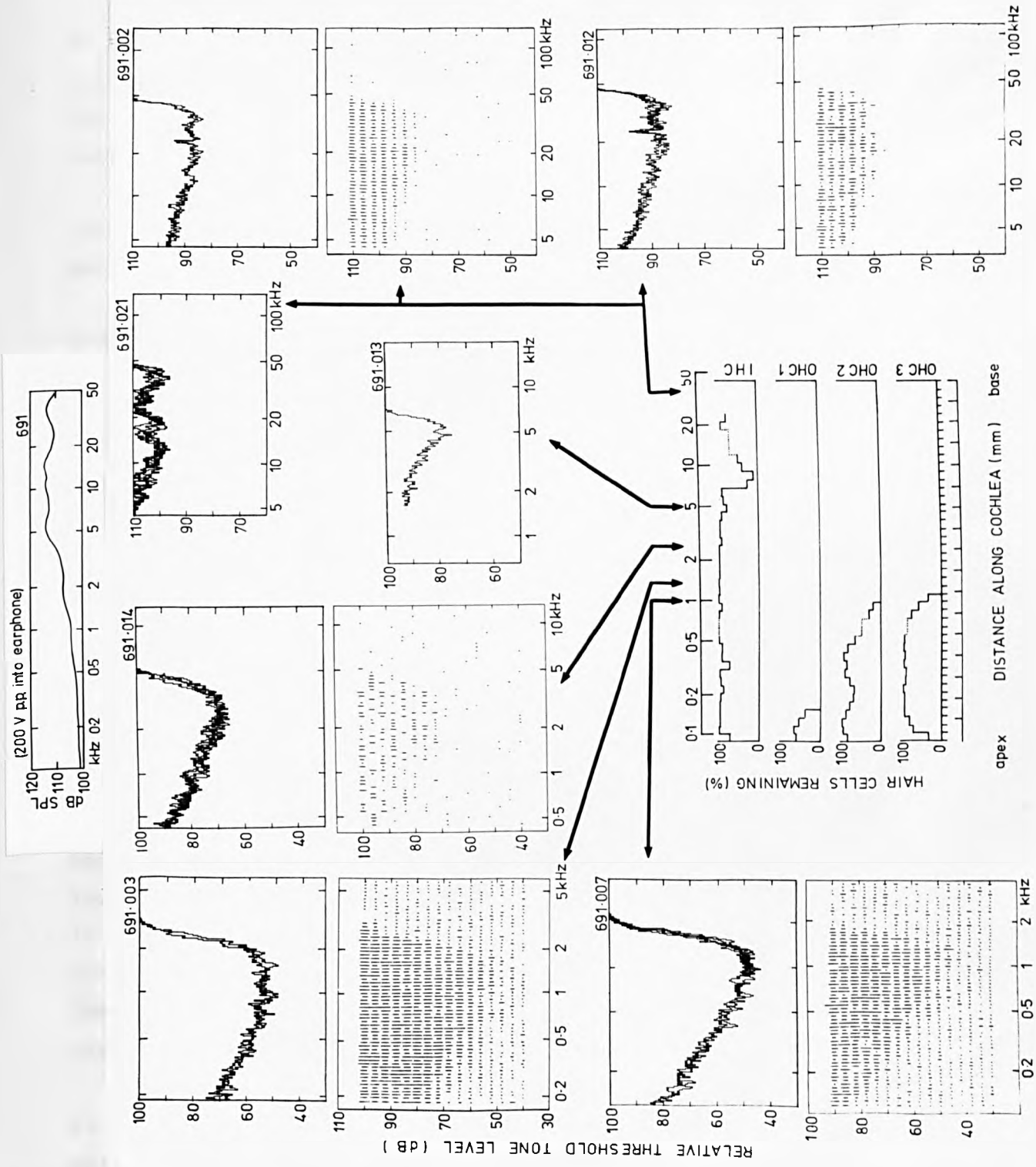


Figure 4.23 The effect of kanamycin induced hair cell loss on cochlear fibre FTCs. A sample of 7 FTCs from a kanamycin treated GP is shown. The FTCs were determined by: a) a threshold following paradigm (upper curves of FTC pairs, see text for details), and b) in five examples shown, by a method in which the fibre is presented with gated pure tone stimuli in a pseudo-random sequence to cover a large area of frequency and intensity space. The fibre's response to each stimulus frequency/intensity combinations is indicated by the length of the appropriate vertical dash. The stimulus levels are given in relative terms of the electrical input to the condenser earphone and they represent approximate (± 6) dB SPL at the tympanic membrane. The variation in stimulus level across frequency is indicated by the plot of dB SPL, at the tympanic membrane, for a 200 v p.p. signal into the condenser earphone. The cochleogram shows the percentage of hair cells remaining in the cochlea at the time of the physiological recording.

The consistent finding was of a deterioration of the tuning properties of cochlear fibres from regions of OHC loss, most obviously in fibres from regions of total OHC loss. Qualitatively, the changes in FTCs could be described as a loss of the low threshold, sharply tuned region of the FTC leaving behind a broadly tuned, high threshold FTC.

In order to assess the changes in cochlear tuning which accompany the loss of OHCs, the FTC can be conveniently characterized in terms of the steepness of its high and low frequency cut-off slopes, and its 10dB bandwidth.

4.4b CHANGES IN FTC BANDWIDTH AFTER OHC LOSS.

The relative sharpness of cochlear fibre tuning is commonly expressed in terms of the $Q_{10\ dB}$ value of the FTC (the CF of the unit divided by bandwidth of the FTC 10 dB above minimum threshold). These values for over 350 fibres are plotted against the characteristic frequency of the fibres in figure 4.24.

The $Q_{10\ dB}$ values of normal cochlear fibres (filled symbols) show an increase in sharpness of tuning with increasing CF of the fibres. This is a feature common to normal fibres in the mammals which have been investigated (see EVANS 1975a for review).

Cochlear fibres from regions of OHC degeneration (open symbols) showed a decrease in $Q_{10\ dB}$ value indicating a broadening of tuning. Fibres from areas of total OHC loss (open circles) have $Q_{10\ dB}$ values of 0.3-3 (CFs 2-22 kHz) compared with normal values of approximately 2-6 at 2 kHz and up to about 4-10 at 20 kHz. Thus the tuning of fibres from regions of total OHC loss is 5-10 times less sharp than normal in terms of their $Q_{10\ dB}$ values. It is worth noting that the $Q_{10\ dB}$ values of such pathological fibres are similar to the equivalent values for the BM mechanical tuning measurements. Such values are indicated in figure 4.24 by the star symbols joined by the dashed line (see figure legend for details).

Fibres from cochlear regions where row 1 OHCs were lost (a common feature in the kanamycin treated cochlea see figure 3.1, cochleograms G-P) also had, on average, lower $Q_{10\ dB}$ values than normal fibres.

The small points in figure 4.24 show that the fibres from control animals which had suffered cochlear hypoxia, often had low $Q_{10\ dB}$ values reflecting modification of the FTC in a manner similar to the changes accompanying OHC loss.

In figures 4.25 & 4.26 respectively, the steepness (in dB/octave) of the high and low frequency cut-off slopes[3] of the FTCs are plotted against the CF.

In normal cochlear fibres (filled symbols) the steepness of the high frequency (HF) cut-off slope increases with increasing fibre CF from approximately 30 dB/octave at 0.5 kHz to 60 dB/octave at 2 kHz. At 10 kHz the majority of fibres have FTCs with HF cut-off slopes of between 150-500 dB/octave,

[3] All cut-off slopes were computed 5-25 dB above minimum threshold.

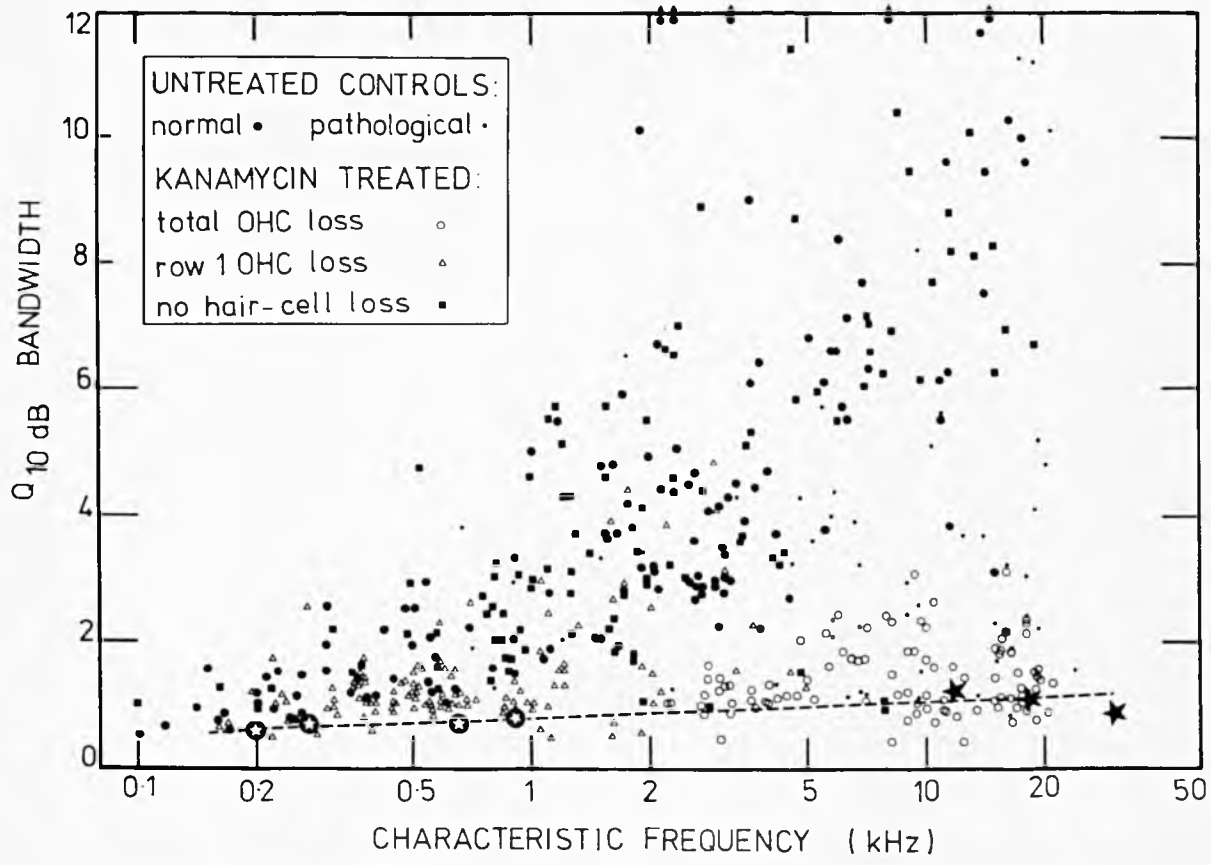


Figure 4.24 The sharpness of cochlear tuning expressed as the $Q_{10\,dB}$ of the FTC for cochlear fibres from normal and kanamycin treated GPs, plotted against the CF of each fibre. The fibres are categorized as indicated in the key. The star symbols (joined by the dashed line; taken from EVANS, 1975a) show the $Q_{10\,dB}$ values for direct basilar membrane tuning measurements: The points below 1 kHz are the data of VON BÉKÉSY (1944) and the filled stars those of WILSON & JOHNSTONE 1972.

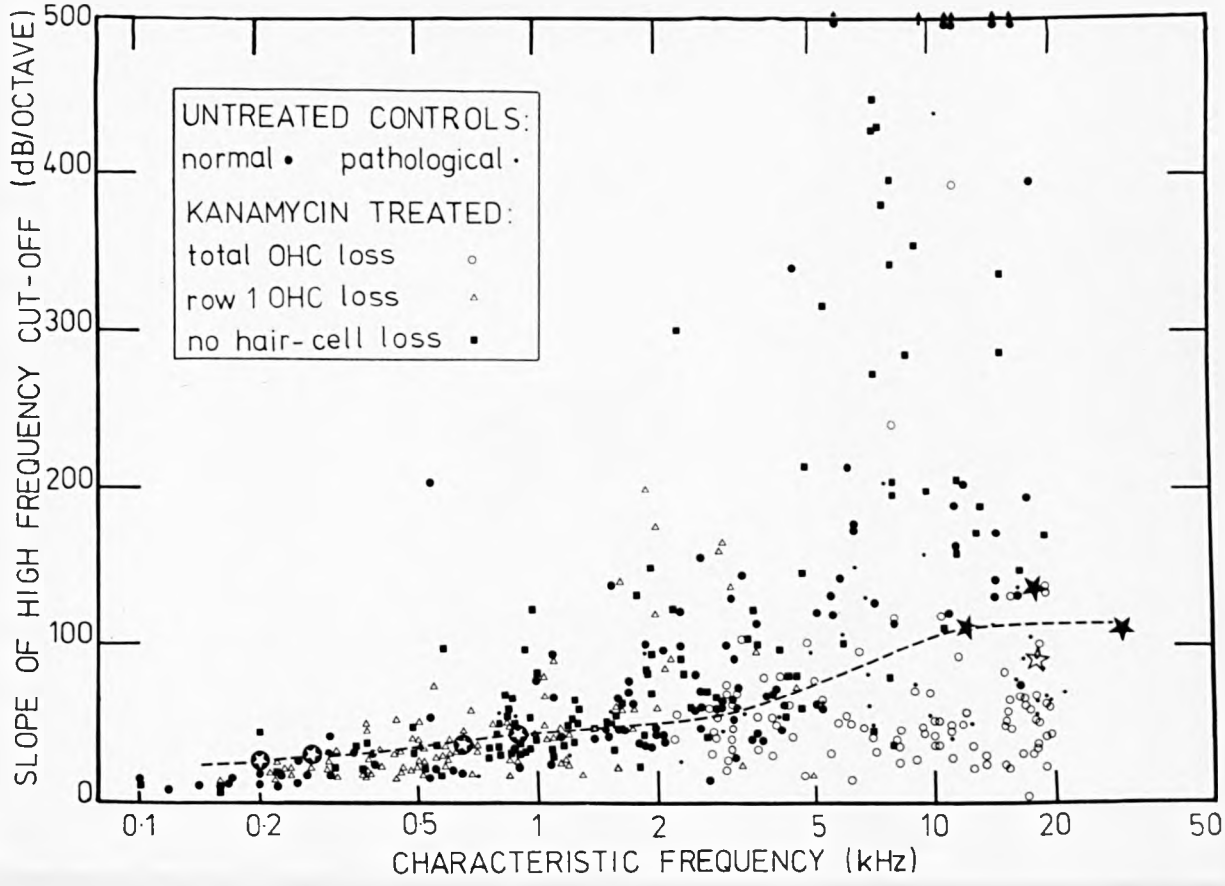


Figure 4.25 The slopes of the high frequency cut-off of cochlear fibre FTCs recorded from normal and kanamycin treated GPs, plotted against the CF of each fibre. The slopes are measured over a region 5-25 dB above the minimum threshold of the fibre. The fibres are categorized as indicated in the key. The star symbols (joined by the dashed line; taken from EVANS 1975a) show analogous HF cut-off slope values for the basilar membrane tuning measurements by VON BÉKÉSY (1944, points below 1 kHz) and by WILSON & JOHNSTONE, 1972 (filled stars).

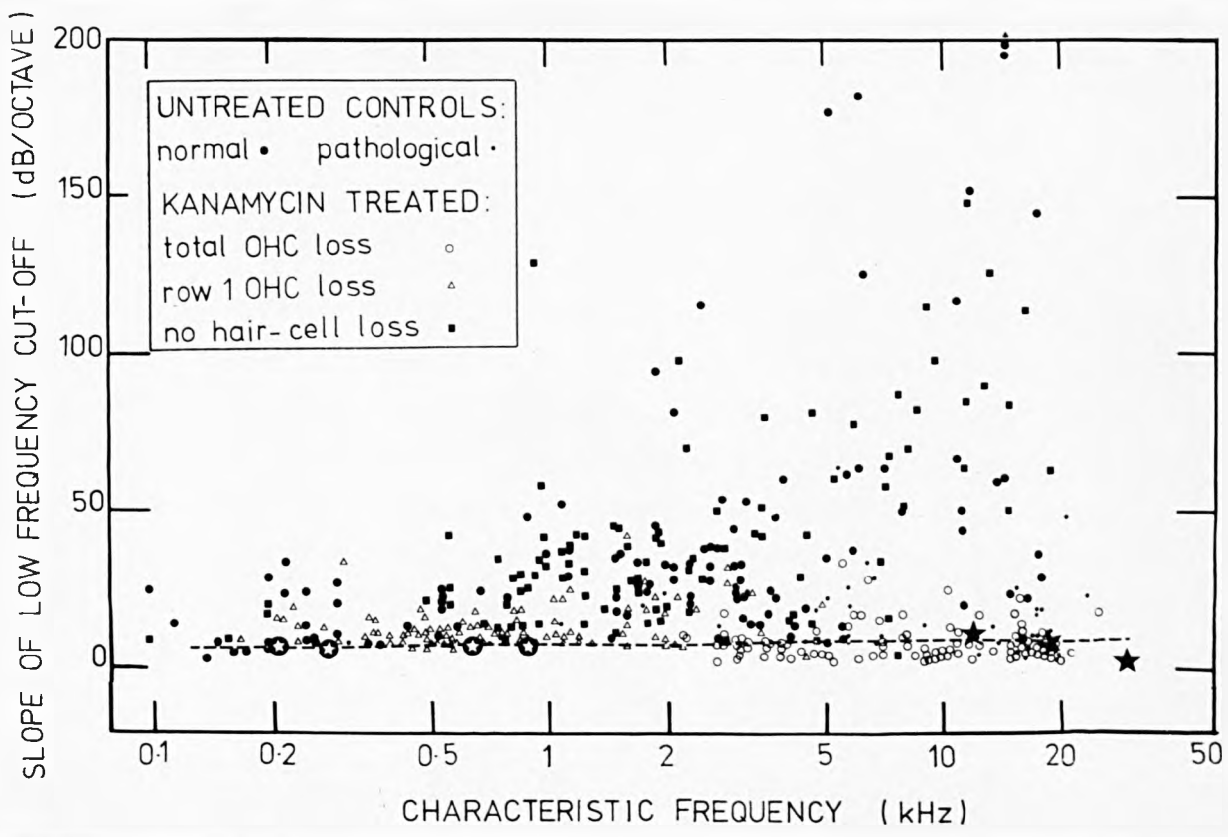


Figure 4.26 The slopes of the low frequency cut-off of cochlear fibres FTCs recorded from normal and kanamycin treated GPs, plotted against the CF of each fibre. The slopes are measured over a region 5–25 dB above the minimum threshold of the fibre. The fibres are categorized as indicated in the key. The star symbols (joined by the dashed line; taked from EVANS 1975a) show analogous LF cut-off slope values for the basilar membrane tuning measurements by VON BÉKÉSY (1944, points below 1 kHz), and by WILSON & JOHNSTONE, 1972 (filled stars).

and sometimes steeper. In fibres from areas of total OHC loss (open circles) the HF cut-off slope of the FTC is reduced to approximately 20-80 dB/octave. The HF cut-off slope of most of these pathological fibres is much less steep than the corresponding HF cut-off slope of the measured BM tuning (star symbols & dashed line in figure 4.25; see legend for details).

The normal low frequency (LF) cut-off slopes (filled symbols) of figure 4.26 increase from approximately 20 dB/octave below 0.5 kHz to 30-40 dB/octave at 2 kHz. For fibres above 2 kHz the low frequency cut-off slopes become much steeper, and can reach 150 dB/octave at CFs around 10 kHz. In fibres from areas of total OHC loss (open circles) the LF cut-off is reduced, often to 1-2 dB/octave - reflecting the change of FTC tuning from a band-pass to almost a low-pass characteristic.

4.4c THE DEPENDENCE OF FTC BANDWIDTH AND CUT-OFF SLOPES ON MINIMUM THRESHOLD ELEVATION.

In figure 4.27 the 10 dB bandwidths (in octaves) of 450 cochlear fibres are plotted against the threshold elevation[4] of the cochlear fibre in dBs. Because threshold and tuning properties of normal cochlear fibres change systematically with CF, correlations between bandwidth and threshold elevation can only be justifiably made on fibres within a restricted range of CFs. In figure 4.27 this was carried out for six octave ranges from 0.5 kHz to 16 kHz and above. The dashed line joins the mean bandwidth values calculated within 20 dB overlapping segments.

For fibres with CFs below about 2 kHz, the bandwidth increased approximately linearly with dB threshold elevation. For fibres with CFs above 2 kHz, the relationship was not linear, and large changes in bandwidth only occurred if the threshold elevation exceeded approximately 40 dB.

Figure 4.28 shows the changes in the HF cut-off slope of cochlear fibre FTCs with various degrees of threshold elevation. The dotted line joins the mean cut-off slope values calculated within 20 dB overlapping segments. There was considerable scatter particularly for the slopes of normal fibres with high CFs. For fibres with CFs above 2 kHz, the HF cut-off slope decreased with threshold elevations greater than 40 dB, by 50-60% (on average). For fibres with low CFs (below 2 kHz) there was very little change in FTC HF cut-off slopes with threshold elevation.

In figure 4.29 the LF cut-off slopes of the FTCs of cochlear fibres are plotted against the threshold elevation of each fibre. It is more difficult

---

[4] Only cochlear fibres with threshold elevation which accompanied OHC loss are under consideration; data from fibres with acute pathology are not included.

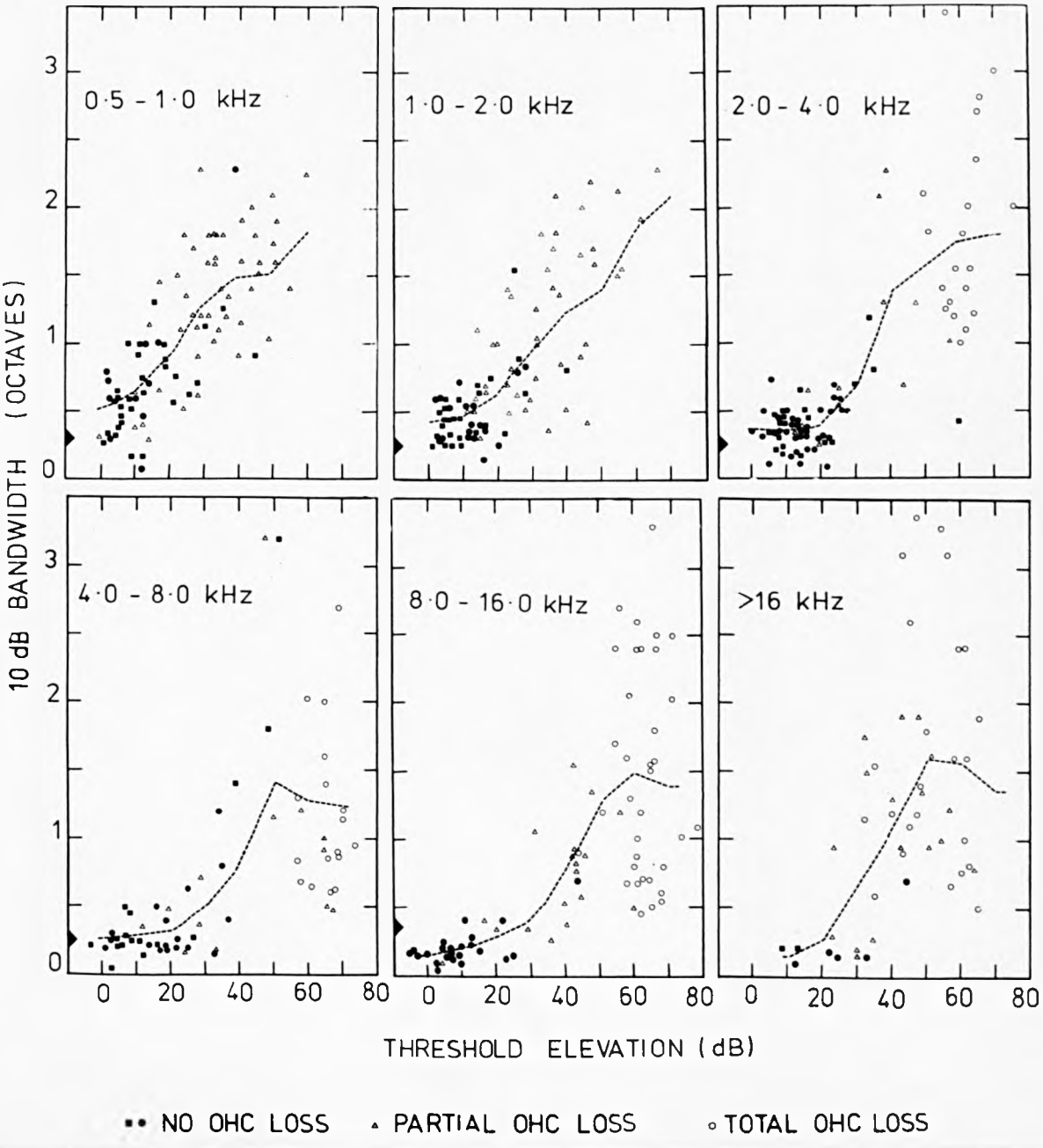


Figure 4.27 The relationship between the bandwidth of cochlear fibre FTCs from normal and kanamycin treated GPs, and the minimum threshold elevation of each fibre. The threshold elevation is measured from a hypothetical normal value based on the most sensitive minimum thresholds found in normal cochlear fibres. Data from 450 fibres from 33 GPs are pooled within octave bands as indicated. The filled symbols indicate fibres from normal cochlear regions (no OHC loss). Open triangles and circles indicate fibres from regions of partial and total OHC loss respectively, but with IHCs intact. The dotted line joins the mean bandwidth values for fibres falling within overlapping 20 dB ranges.

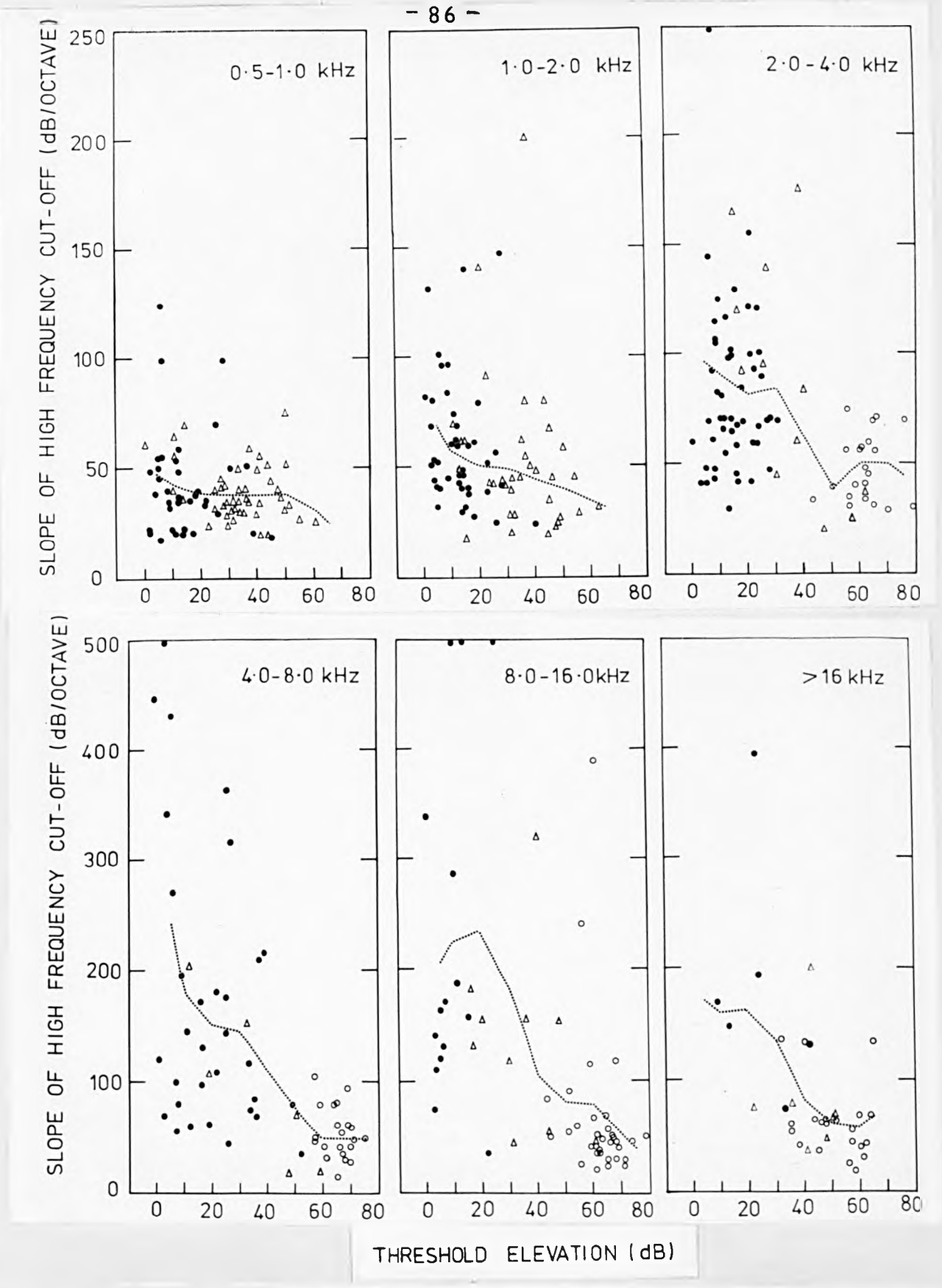


Figure 4.28 The relationship between the high frequency cut-off slope of cochlear fibre FTCs (5-25 dB above minimum threshold) from normal and kanamycin treated GPs, and the minimum threshold elevation of each fibre. The threshold elevation is measured from a hypothetical normal value based on the most sensitive minimum threshold found in normal cochlear fibres. Data from 450 fibres from 33 GPs are pooled within octave bands as indicated. The filled symbols indicate fibres from normal cochlear regions (no OHC loss). Open triangles and circles indicate fibres from regions of partial and total OHC loss respectively, but with IHCs intact. The dashed line joins the mean cut-off slope values for fibres falling within overlapping 20 dB ranges.

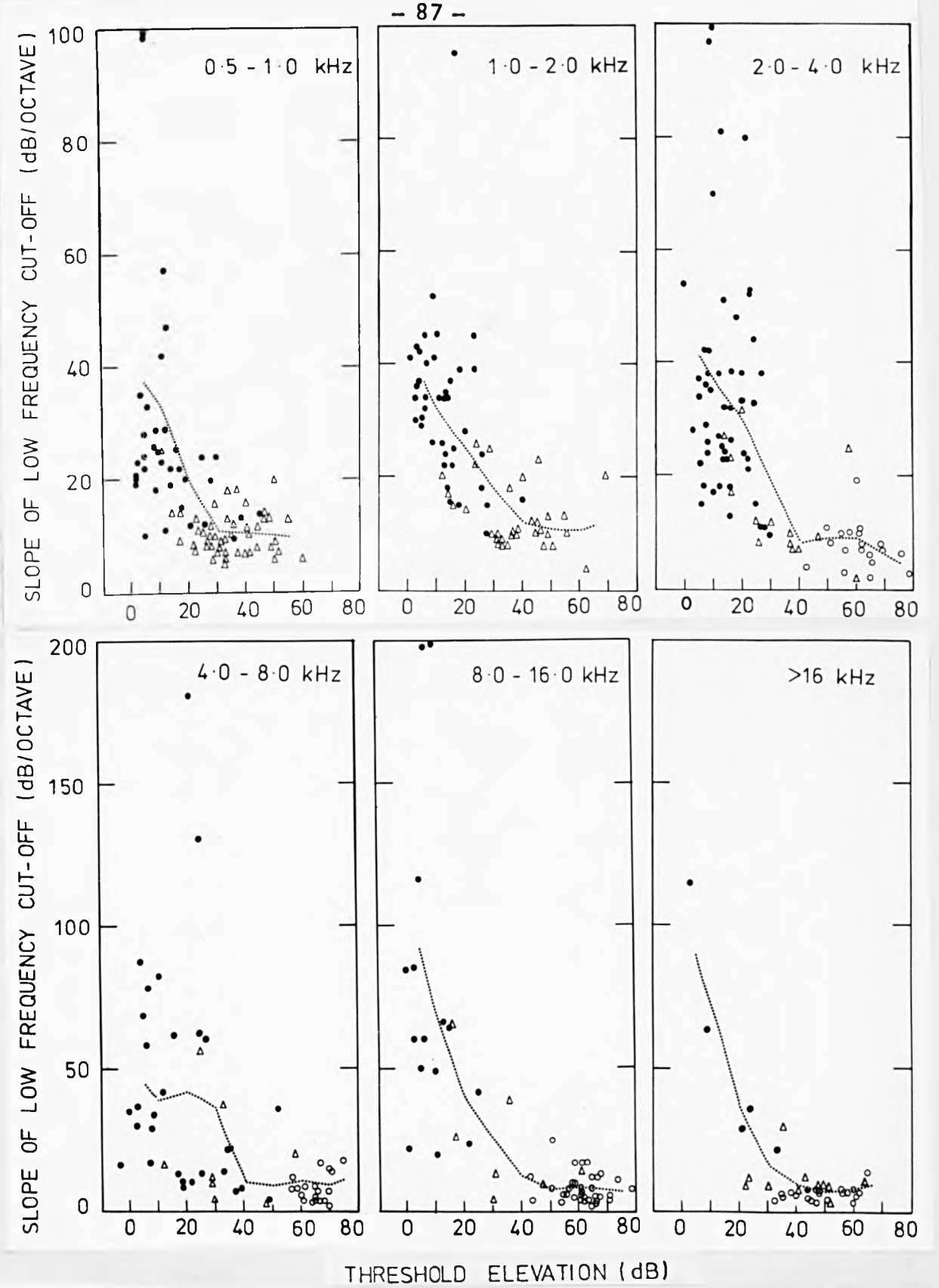


Figure 4.29 The relationship between the low frequency cut-off slope of cochlear fibre FTCs (5–25 dB above minimum threshold) from normal and kanamycin treated GPs, and the minimum threshold elevation of each fibre. The threshold elevation is measured from a hypothetical normal value based on the most sensitive minimum thresholds found in normal cochlear fibres. Data from 450 fibres from 33 GPs are pooled within octave bands as indicated. The filled symbols indicate fibres from normal cochlear regions (no OHC loss). Open triangles and circles indicate fibres from regions of partial and total OHC loss respectively, but with IHCs intact. The dashed line joins the mean cut-off slope values for fibres falling within overlapping 20 dB ranges.

to generalize about the changes in the LF cut-off slope from this figure because the LF cut-off slope of FTCs from normal cochlear fibres is made up of two sections. Near to the tip of the normal FTC the LF cut-off is relatively steep. At high intensity it flattens out and forms the FTC tail. The slope of the low frequency slope of the FTC is computed 5-25 dB above minimum threshold and thus for low threshold fibres, the computed slope will be the steep slope near the FTC tip. For fibres of increasingly elevated thresholds, the computed slope will increasingly reflect the low slope of the tail. The point of inflexion in the mean curves (dotted lines) of figure 4.29 indicates that loss of any steep component to the LF cut-off occurs in fibres with threshold elevation of approximately 40 dB.

Returning to a more qualitative description of the changes in FTC, with threshold elevation, figure 4.30 shows a sample of FTCs from normal and kanamycin treated GPs, grouped according to their CF. The scale for individual FTCs is shown above the figure, and the FTCs are positioned vertically according to the elevation of their minimum thresholds (indicated on the adjacent scale). The changes could well be described as a gradual shortening of the sharply tuned tip segment of the FTC until at 40-50 dB of threshold elevation, only a broadly tuned FTC remains.

The results of the cochlear fibre studies reported in this chapter are briefly discussed in chapter 7.

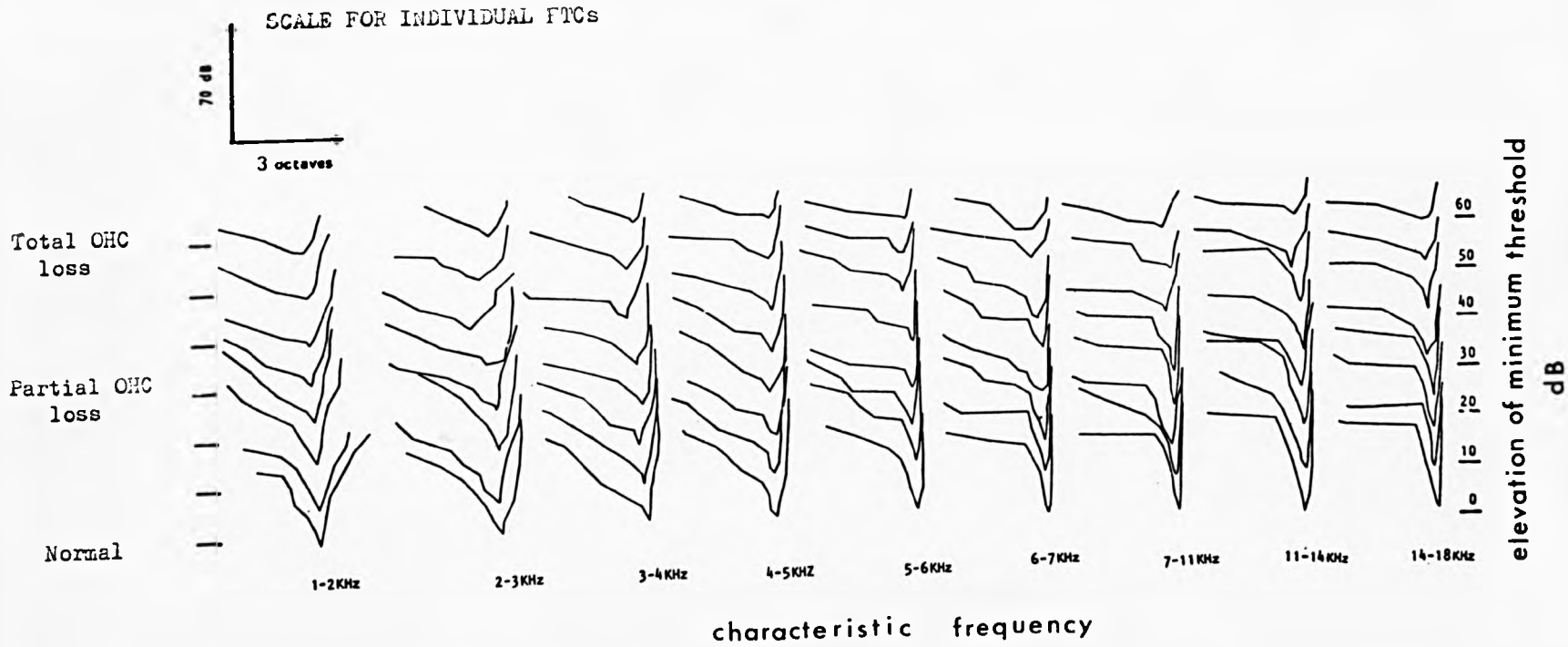


Figure 4.30 A qualitative illustration of the changes in FTC which accompany increasing degrees of OHC loss. A sample of 64 cochlear fibre FTCs from normal and kanamycin treated GPs are grouped according to the fibre CF. Each FTC is plotted on the scale shown (top left of figure). For clarity, the CFs of the FTCs are displaced vertically and positioned according to the elevation of their minimum thresholds(indicated on the right hand scale).

CHAPTER 5.

RESULTS: COCHLEAR ACTION POTENTIAL (CAP) STUDIES.

5.1 CAP THRESHOLDS TO FREQUENCY SPECIFIC (TONE PIP) STIMULI.

5.2 CAP AMPLITUDE & LATENCY : INTENSITY FUNCTIONS IN NORMAL AND PATHOLOGICAL GUINEA PIG COCHLEAS.

5.2a CAP AMPLITUDE & LATENCY : INTENSITY FUNCTIONS IN NORMAL COCHLEAS.

5.2b CAP LATENCY : INTENSITY FUNCTIONS IN PATHOLOGICAL COCHLEAS.

The secondary aim of the present study was to investigate how CAPs are related to single cochlear fibre recordings in the same animal. Two types of CAP measurement have been investigated:

a) The thresholds of CAP responses to frequency specific (tone pip) stimuli, and

b) the amplitude : intensity and latency : intensity functions.

5.1 CAP THRESHOLDS TO FREQUENCY SPECIFIC (TONE PIP) STIMULI.

The question asked was: how far can CAP thresholds serve as an indication of individual cochlear fibre thresholds in the normal cochlea, and under various conditions of hair cell loss?

The CAP thresholds (determined by visual detection of the response of appropriate latency on the oscilloscope trace) to tone pip stimuli, have been measured in 48 GPs (36 kanamycin treated animals and 12 normal controls). In each animal the CAP thresholds were determined at 10-20 frequencies in the range 0.5-40 kHz, thus obtaining the CAP audiogram. The tone pip stimuli were of 4 ms duration, 2 ms rise/fall times (methods section 2.7a).

The usefulness of the CAP audiogram for indicating the threshold of response across the frequency range has been investigated most directly, by comparison of the CAP threshold with the minimum thresholds of single cochlear fibres. A less direct comparison could also be made between CAP threshold elevations and the corresponding degree of hair cell loss in the cochlea, because the approximate minimum threshold elevation of cochlear fibre responses can be inferred from the percentage of hair cell loss in the cochlear region from which they originate (section 4.3b; figure 4.8).

Figure 5.1 shows the CAP audiograms (dotted curve joining filled circles) for two normal control GPs. Superimposed, for comparison, are the FTCs from a representative sample of cochlear fibres from the same animal. In figures 5.2-5.9, the CAP audiograms (dotted curves) for eight kanamycin treated GPs are compared with the FTCs of cochlear fibres from the same animals. The cochleograms show the % of hair cell loss in each cochlea from which the CAP & FTCs were obtained. For both normal and pathological cochleas, the CAP thresholds reflect the minimum thresholds of cochlear fibres relatively well, down to frequencies of about 1kHz (see next paragraph for exact relationship). Furthermore, the CAP audiogram indicates the extent and degree of the cochlear lesion. Thus, where there is severe or total OHC loss, the CAP audiogram is elevated some 40-60 dB. Where extensive regions of total hair cell loss occur (inner & outer) the cut-off of the CAP audiogram clearly marks the extent of the lesion, for example the basal 5 mm of 606 (figure 5.8). Even the presence of small areas of residual and functioning hair cells is clearly revealed by the CAP audiogram (figure 5.9).

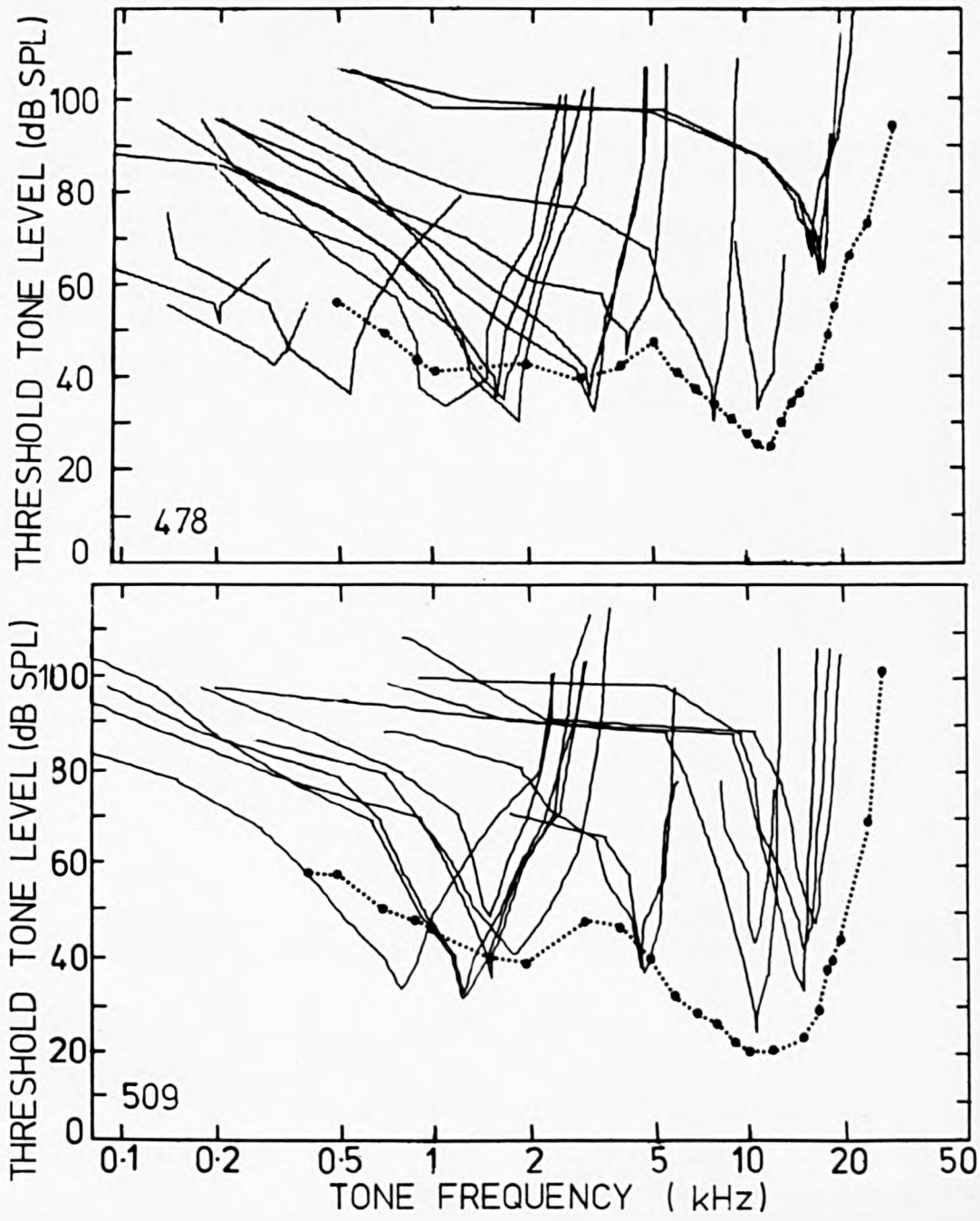
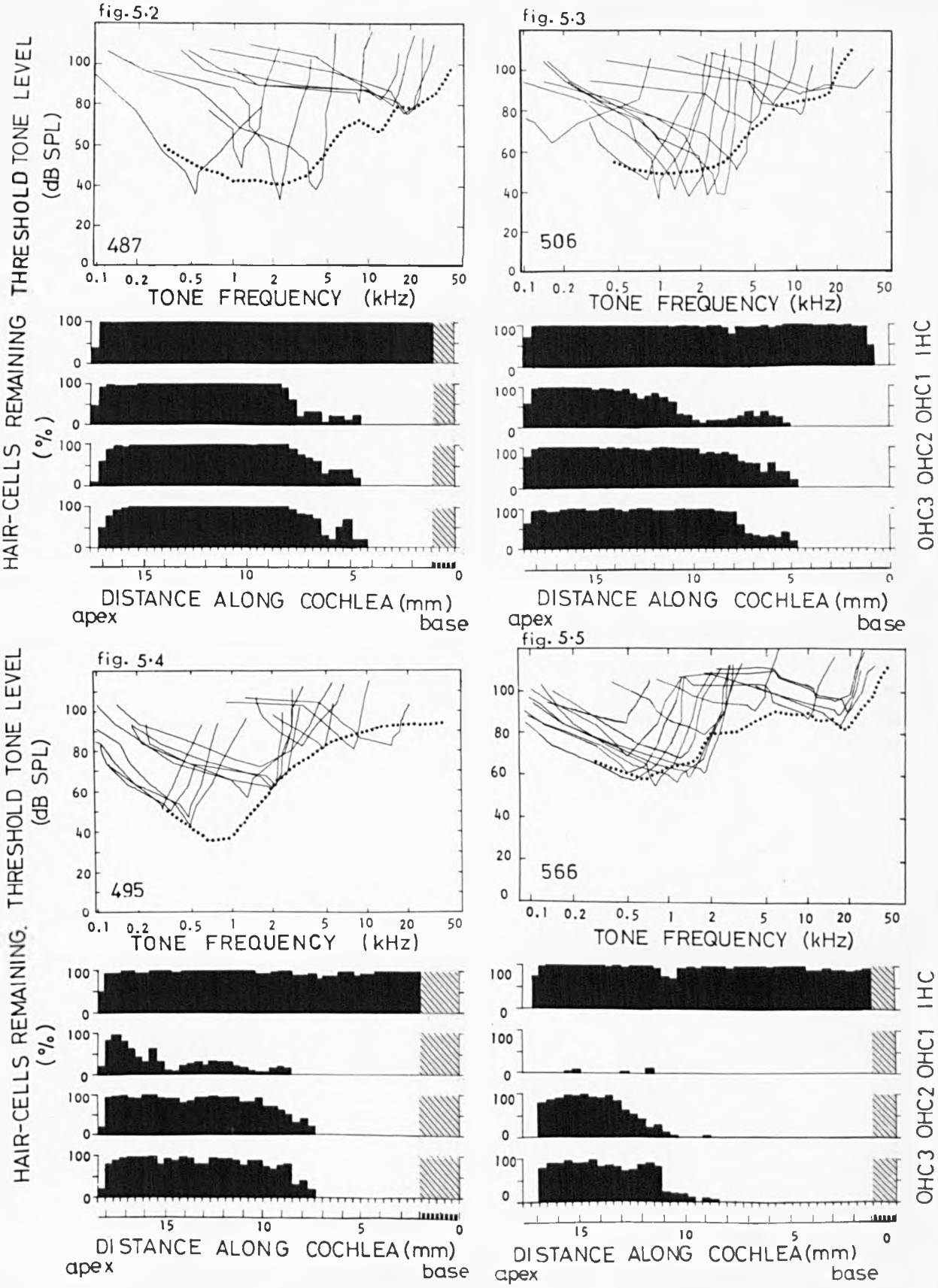
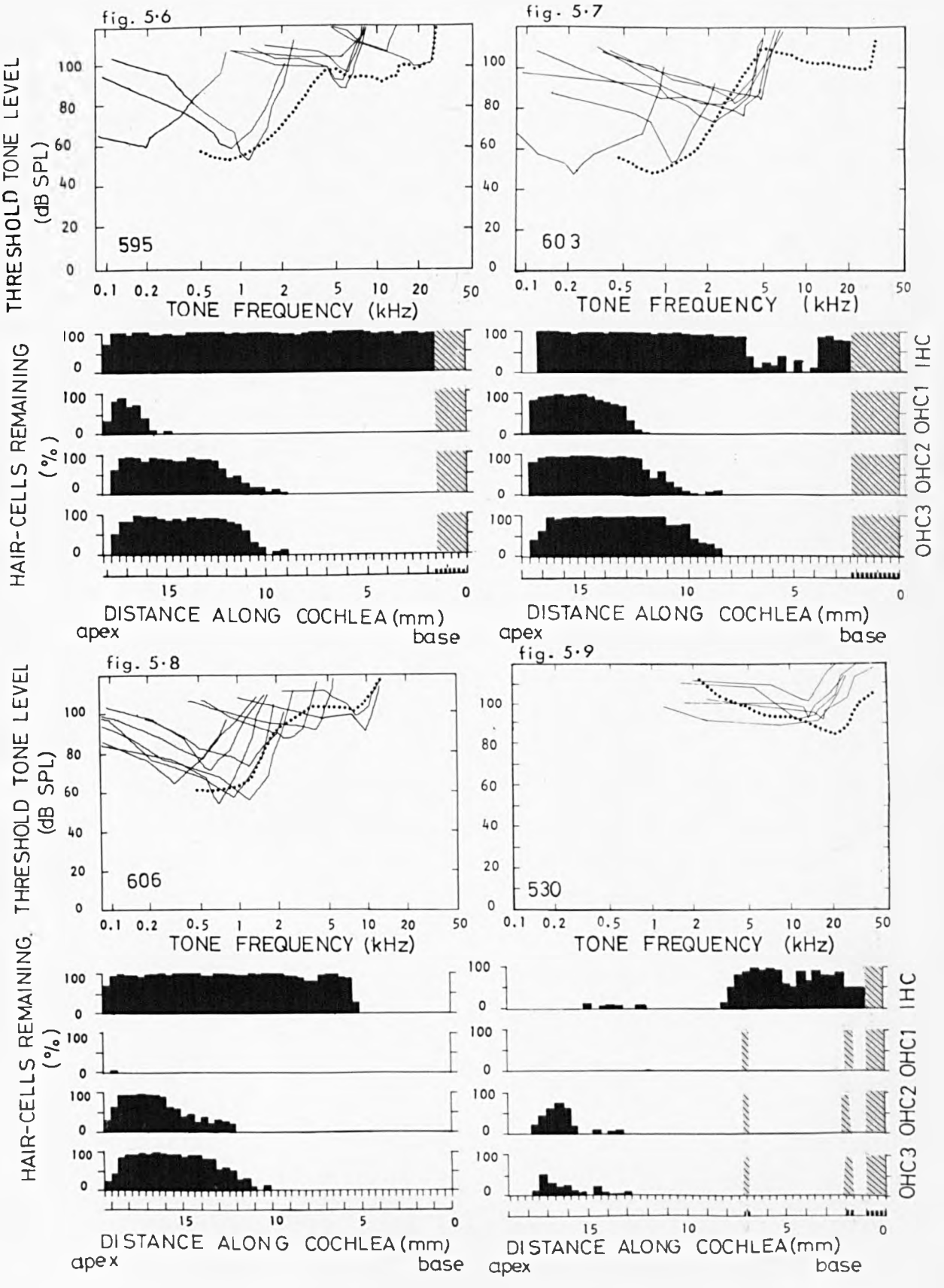


Figure 5.1 A comparison of the CAP audiogram(dotted curve joining filled circles) and cochlear fibre FTCs (continuous curves) in two normal GPs. Thresholds measured in dB SPL at the tympanic membrane.



Figures 5.2 - 5.5 CAP audiograms (dotted curves) compared with cochlear fibre FTCs in four kanamycin treated GPs. Thresholds measured in dB SPL at the tympanic membrane.

The cochleograms indicate the percentage of hair cell in each cochlea from which the CAP audiogram and FTCs were determined. The striped region in some cochleograms indicates a region of the cochlea not examined histologically.



Figures 5.6 - 5.9 See figures 5.2 - 5.5 for legend.

In an attempt to define more exactly the relationship between the single fibre minimum thresholds and the corresponding CAP threshold, the deviation of the single fibre thresholds from the CAP audiogram as a function of frequency has been plotted in figure 5.10. There is some scatter in the data, with 66% of the minimum thresholds within 10dB, and 95% within 20 dB of the CAP audiogram. There are also two trends apparent in the data. At low frequencies (below 2-3 kHz) the CAP thresholds are often higher than single fibre minimum thresholds, and at high frequencies the reverse is often the case, i.e. cochlear fibres have higher minimum thresholds than the measured CAP threshold. These results are discussed in section 8.1.

The CAP audiogram is also useful for monitoring acute changes in cochlear function such as those which could occur during the course of an experiment. For example, manipulation of the brain stem during electrode placement in the cochlear nerve will sometimes interfere with the cochlear blood supply causing cochlear hypoxia. Figure 5.11 illustrates how the CAP audiogram can reflect, across frequency, threshold elevation caused by such cochlear hypoxia. Acute changes in the CAP audiograms for three GPs are shown. The initial CAP audiogram is indicated by the dashed curve, the final one by the continuous curve. The dotted curves in 543 & 520 are CAP audiograms determined during the course of the cochlear deterioration. The filled symbols are the minimum thresholds of cochlear fibres which were determined subsequent to the cochlear deterioration and therefore correspond more closely to the final CAP audiogram.

The threshold of a click evoked CAP[3] was measured immediately before or after plotting the CAP audiograms of figure 5.11, and is indicated by the arrows to the right of each diagram (threshold level in peak equivalent[4] dB SPL). The threshold elevation of the click evoked response matched the threshold elevation of the lowest threshold values of the CAP audiogram. This is not surprising, and is evidence that the CAP threshold to a broadband stimulus is primarily determined by the most sensitive region of the audiogram.

---

3 50 μs duration click; threshold criterion as for CAP audiogram.

4 equivalent to peak value of a 10 kHz signal.

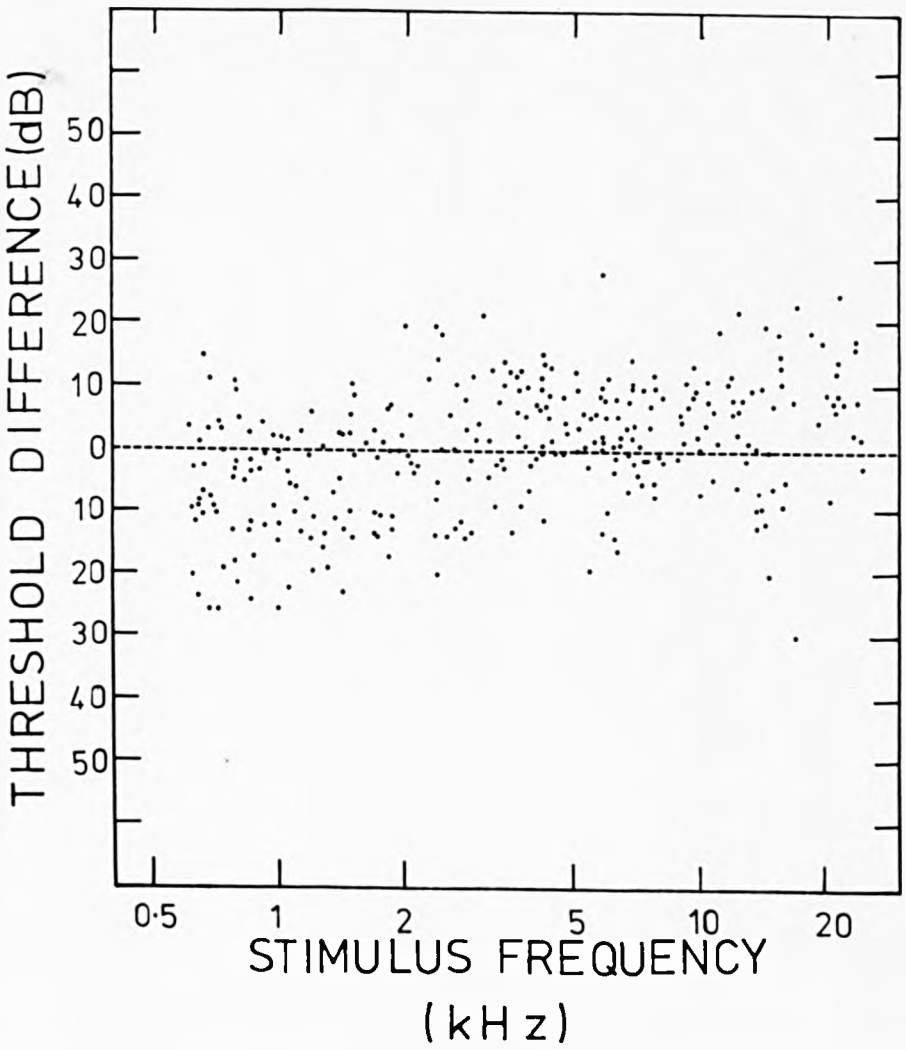


Figure 5.10 The deviation (dB) of the minimum thresholds of cochlear fibres (filled symbols) from the CAP thresholds (dashed line) plotted against stimulus frequency.

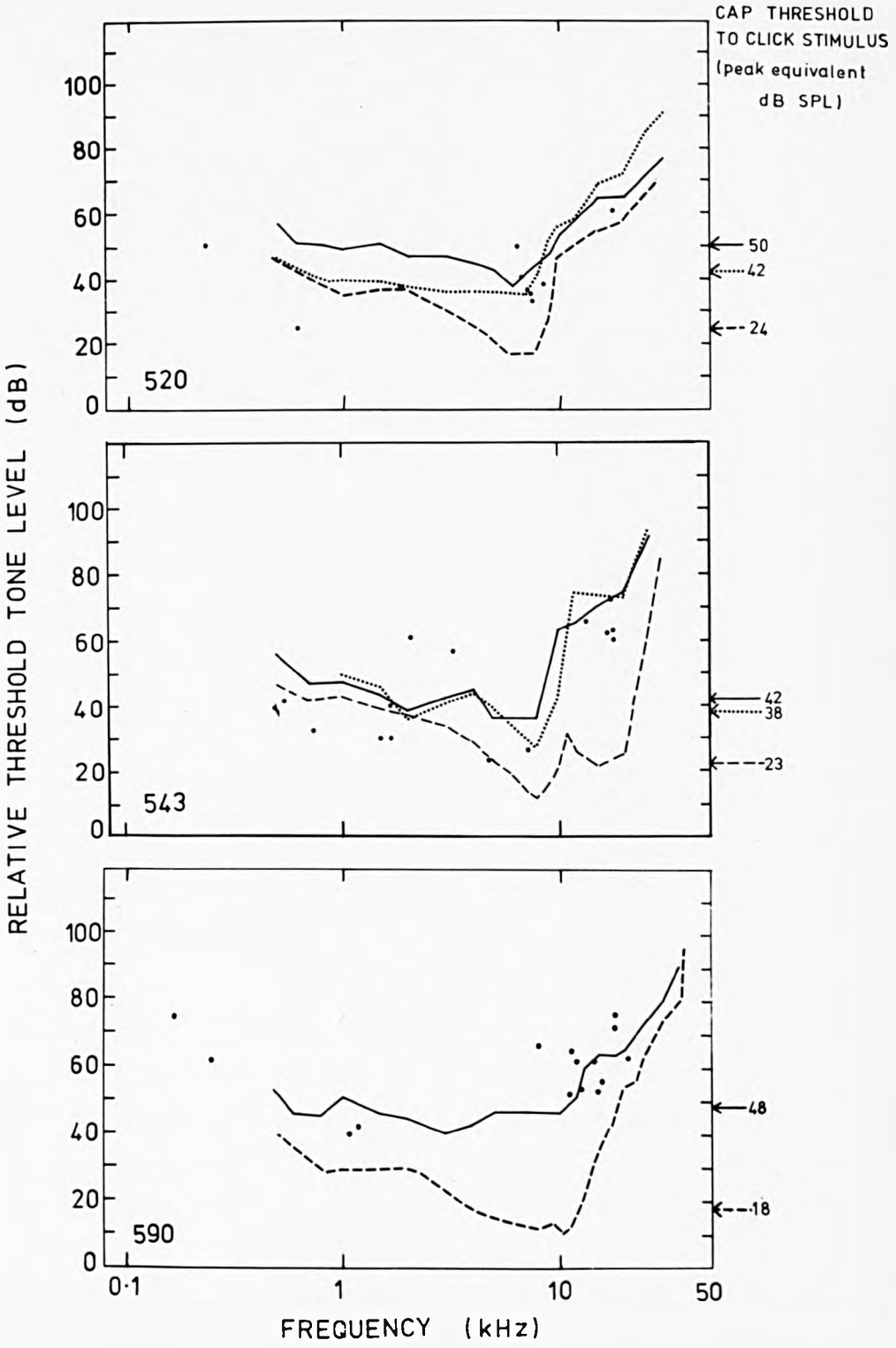


Figure 5.11 CAP audiograms from three GPs, measured before (dashed curves) and after (continuous curves) cochlear hypoxia. (The dotted curves of 543 & 520 are CAP audiograms determined during the deterioration). To the right of each figure are indicated the CAP thresholds to a click stimulus (50 μs) measured immediately before or after the corresponding CAP audiogram (threshold level in peak equivalent dB SPL). The filled circles are the minimum thresholds of cochlear fibres determined after the acute cochlear hypoxia.

5.2 AMPLITUDE AND LATENCY : INTENSITY FUNCTIONS IN NORMAL AND PATHOLOGICAL GUINEA PIG COCHLEAS.

The purpose of this study on amplitude and latency : intensity functions was two fold (for details see introduction section 1.1b). In brief it was aimed:

a) to test some models of the origin of the shape of the amplitude and latency : intensity functions, particularly predictions of those models with regard to the shape of the functions in cochlear pathology and:

b) to determine which features (if any) of such functions are useful indicators of the degree or extent of a cochlear lesion.

To these ends, the functions have been measured in normal GP cochleas and those with lesions caused by kanamycin poisoning, and the main characteristics of these functions (e.g. maximum amplitude, threshold, steepness of slope) are here defined.

5.2a AMPLITUDE AND LATENCY : INTENSITY FUNCTIONS IN NORMAL COCHLEAS.

Figure 5.12 shows the amplitude and latency : intensity functions of the first negative going peak (N_1) of the CAPs evoked by unfiltered click stimuli. The results are from eight control GPs. Each data point of the function is an average of 50-200 responses. The rate of stimulus presentation was 5/s; the use of a 200 ms inter-stimulus interval (ISI) avoided any response equilibration.[6] In the functions of figure 5.12, the amplitude is expressed as a percentage of the CAP amplitude obtained with the maximum stimulus level. This amplitude is indicated on each function and for normal cochleas was approximately 0.5 mV (there was some variability, 0.3-0.68 mV). The amplitude of the CAP increased almost linearly with increasing stimulus intensity expressed in dB. Only the cochleas with slight (6-10 dB) elevation in CAP threshold (543 & 613) could be described as having amplitude functions made up of two segments. In these cases, the CAP amplitude grew slowly at low intensities, and much more rapidly at high intensities.

---

[6] equilibration (often termed cochlear adaptation) is the decrease in amplitude of the CAP response with decrease in ISI. 120 ms is the smallest ISI for which no equilibration occurs in GP (EGGERMONT & ODENTHAL, 1974).

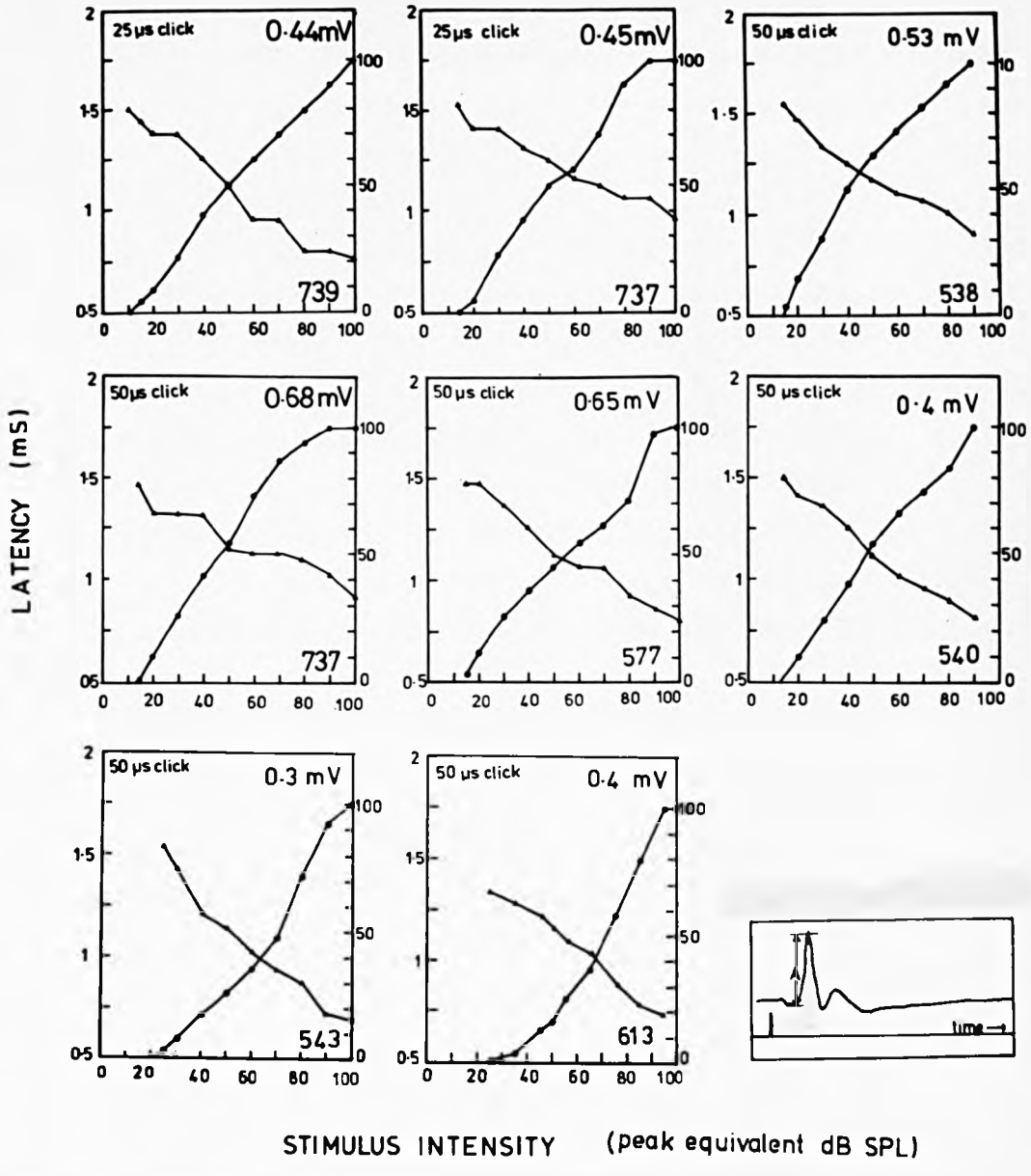


FIGURE 5.12 Click evoked CAP amplitude and latency : intensity functions from eight control GPs. The click stimulus duration was 25 or 50 µs as indicated. The amplitude measurement was measured from the averaged waveform as depicted in the lower right hand diagram. The latency of the CAP (N_1) was measured from the electrical pulse wave-form and then corrected for acoustic delay of 0.09 ms. The CAP amplitude is plotted as a percentage of its response to maximum stimulation (the absolute amplitude values are indicated). The stimulus intensity is given in peak equivalent terms, re. a 10 kHz pure tone. The recording electrode was positioned on the cochlea, near to the round window. The indifferent electrode was on neck musculature.

Figure 5.13 shows CAP amplitude : intensity functions from five normal cochleas using tone pip stimuli (4 ms duration, 2 ms rise/fall times). Each data point is an average of 50-200 responses. In contrast to the linear functions (intensity in dB) for click stimuli, the normal amplitude: intensity functions using frequency specific stimuli had curves with more definitely steeper slopes at high stimulus intensity than at low intensities. The maximum amplitude of the tone evoked CAP was smaller than the maximum click evoked CAP. With the stimulus used in this study (2 ms rise time) the maximum amplitude was about 0.2 mV.

For figure 5.12, the latency of the click evoked CAP (N_1) was measured from the peak of the electrical pulse wave-form, and has been corrected for an acoustic delay of 0.09ms. The latency: intensity functions were similar between animals, and indicated a CAP latency near threshold of approximately 1.5 ms, which decreased at maximum stimulation to less than 1.0 ms. The two cochleas of figure 5.12 with slight threshold elevations (543 & 613, bottom row) had significantly shorter CAP latencies at maximum stimulus intensity. The latencies of CAP responses to tone pips were not measured because, depending on the stimulus intensity, the CAP will be initiated at a different (unknown) point on the stimulus envelope rise time.

5.2b AMPLITUDE AND LATENCY : INTENSITY FUNCTIONS IN PATHOLOGICAL COCHLEAS.

The result of severe OHC loss on the amplitude and latency : intensity functions is illustrated in figures 5.14-5.18. The pattern and degree of hair cell loss is indicated by the appropriate cochleogram .

The click evoked amplitude : intensity function in figure 5.14 has threshold and maximum amplitude value within the normal range despite the total OHC loss in the basal 3-4 mm of the cochlea. In the more severely damaged cochleas of figures 5.15 and 5.16, the click evoked amplitude functions show somewhat reduced maximum amplitudes (0.1 mV & 0.075 mV) and a 40-50 dB threshold elevation. However, the value of maximum amplitude is extremely variable between cochleas which have very similar patterns of hair cell loss. Thus in figure 5.18, cochleas 551, 548, 566, 572, & 568 have similar OHC losses, but the maximum amplitudes vary from 0.5 mV- 0.07 mV. As a consequence of this variation, the slope of such functions may or may not be steeper than normal functions at the same stimulus intensities. This is clearly illustrated in figure 5.18: cochleas 551 & 572 have steep amplitude: intensity functions (10-20 μV/dB), whereas functions from similarly damaged cochleas 566 and 568 have much less steep slopes (2-3 μV/dB).

In figure 5.14, the amplitude : intensity function for the 15 kHz stimulus is typical of those functions in which the stimulus frequency corresponds

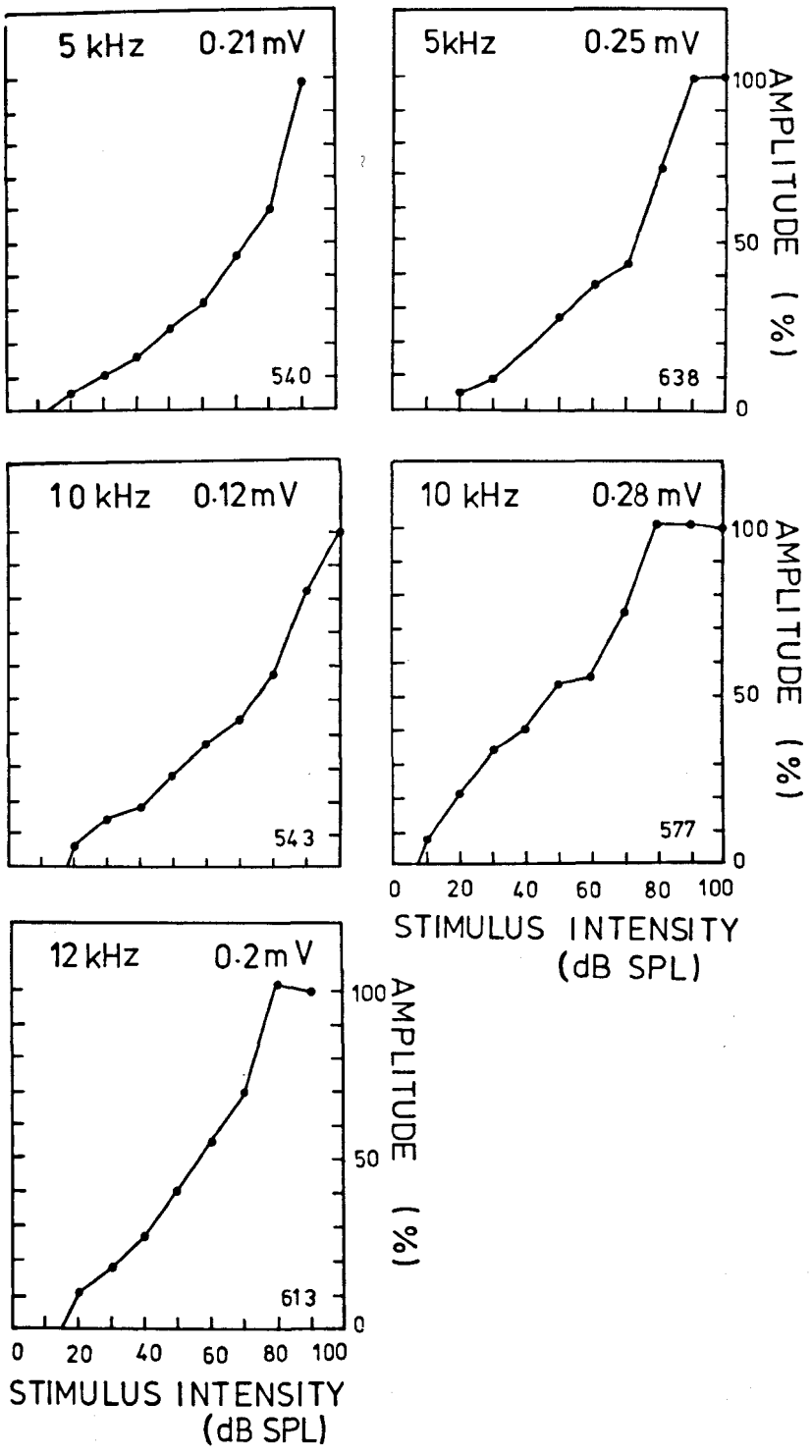


FIGURE 5.13 Tone pip evoked CAP amplitude : intensity functions of five control GPs. The stimulus was a 2 ms rise/fall gated tone (frequency as indicated). The CAP amplitude is plotted as a percentage of its response to maximum stimulation (the absolute amplitude values are indicated). The stimulus intensities indicated are in dB SPL ± 5 dB. The recording electrode was positioned on the cochlea, near to the round window. The indifferent electrode was on neck muscles.

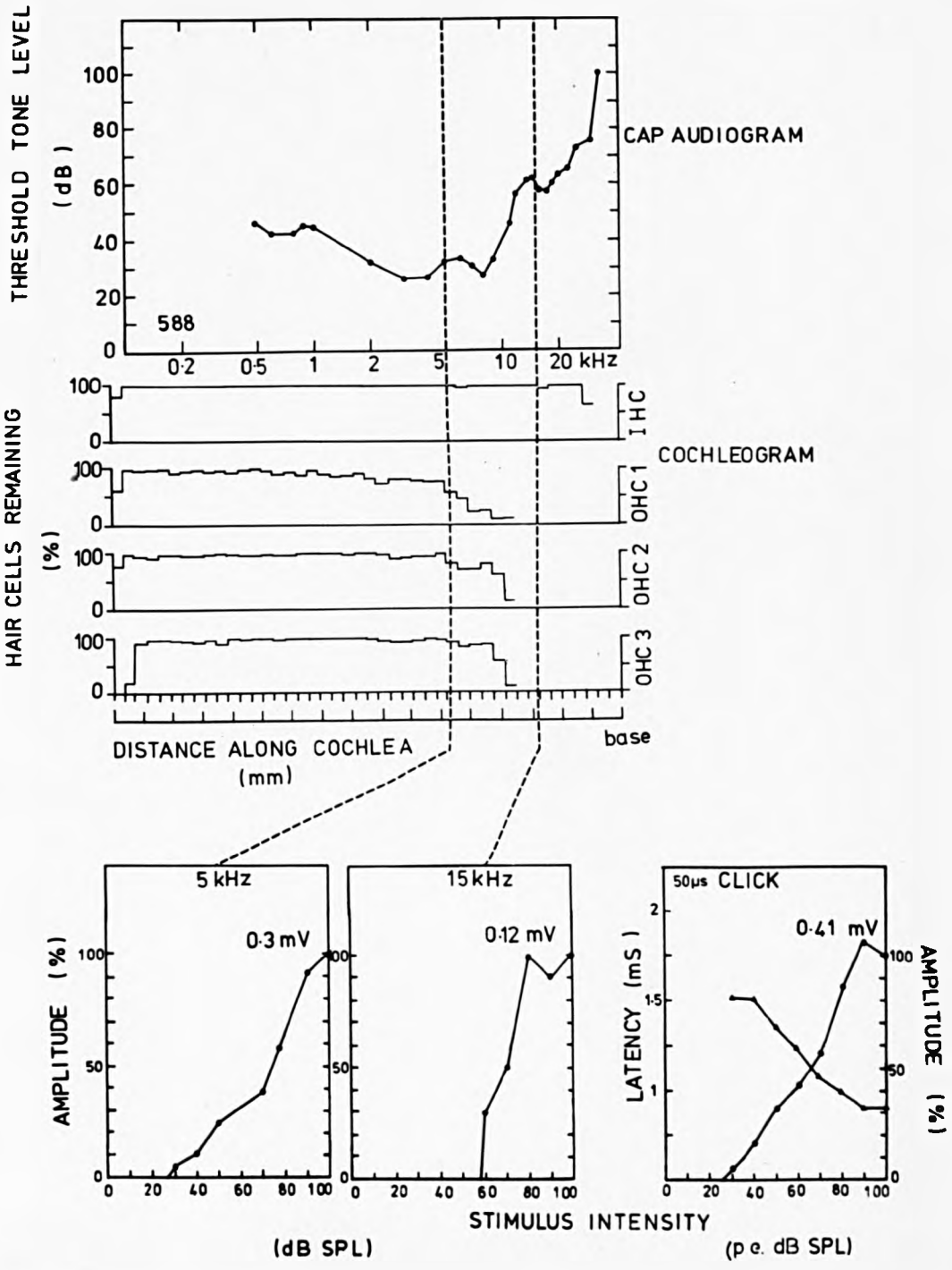


FIGURE 5.14 Tone pip (2 ms rise/fall times) evoked CAP amplitude: intensity functions, and click (50µs) evoked amplitude and latency : intensity functions from a kanamycin poisoned GP.

The cochlear lesion is indicated by the cochleogram and in the CAP audiogram in the upper section of the figure. The amplitudes of the CAP responses are expressed as the percentage of their responses to maximum stimulation. The stimulus intensities indicated are in dB SPL ± 5 dB. For the click stimulus, intensity is expressed in peak equivalent terms re. a pure tone at 10 kHz. The recording electrode was positioned on the cochlea, near to the round window. The indifferent electrode was on neck muscles.

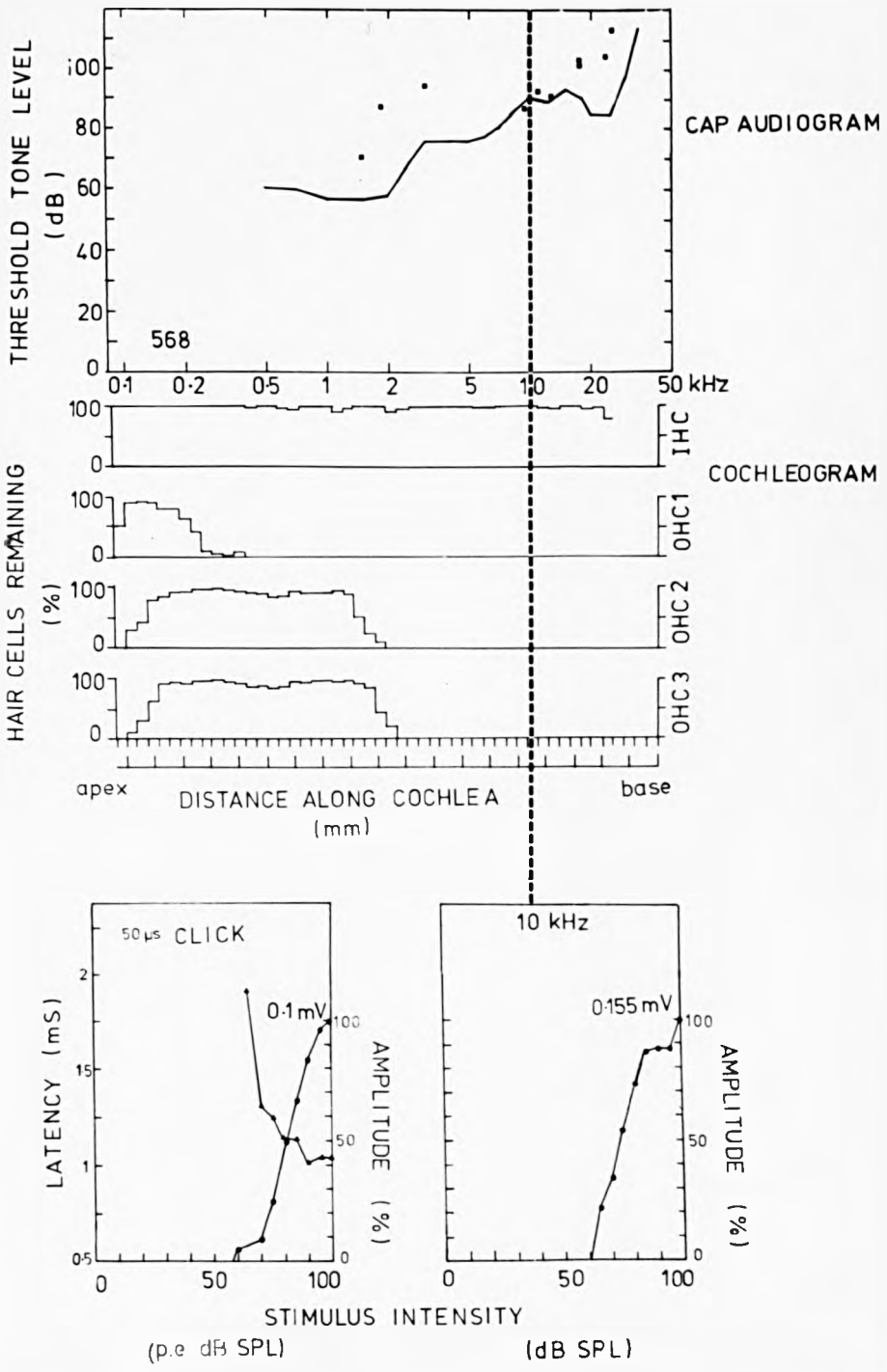


Figure 5.15 see figure 5.14 for legend.

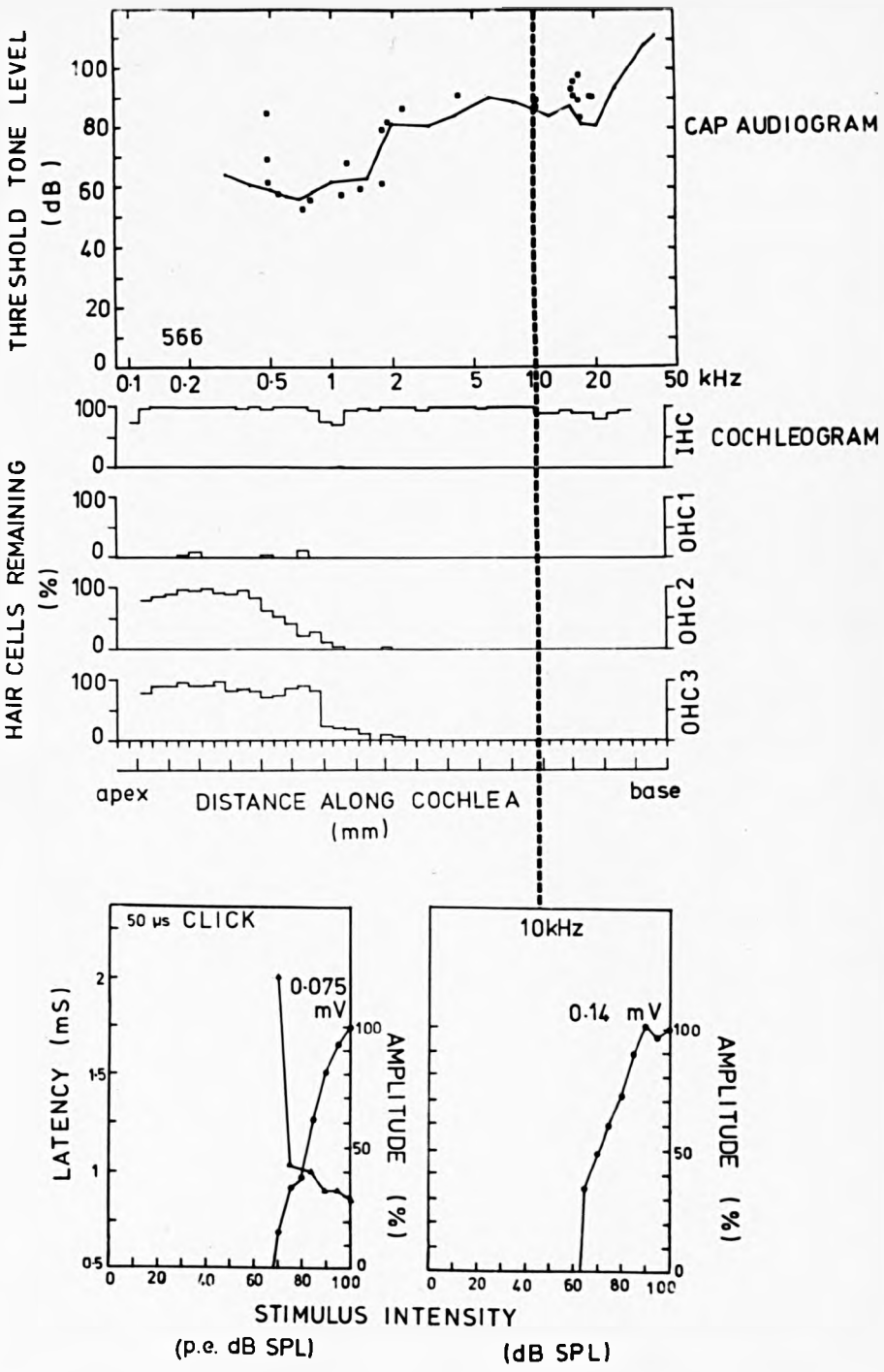


Figure 5.16 See figure 5.14 for legend.

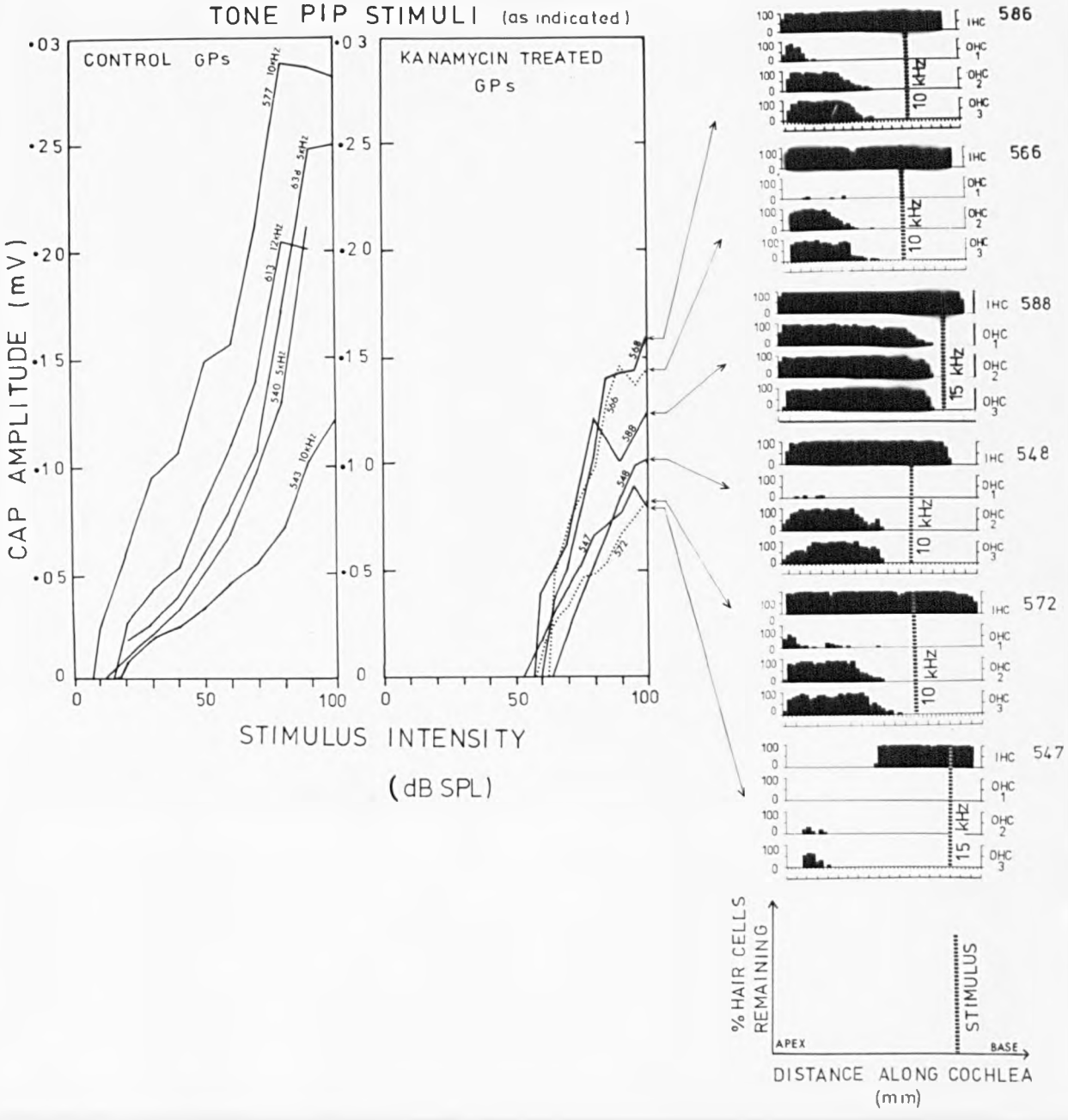


FIGURE 5.1/ Tone pip evoked CAP amplitude : intensity functions for five normal control GPs (left) and six kanamycin treated GPs (right). The stimulus was a 4 ms tone pip (2 ms rise/fall times) at the frequency indicated. The cochleograms on the right indicate the extent of the cochlear lesion when the amplitude : intensity functions were determined. The amplitude of these functions are plotted absolutely, in mV. The stimulus intensities indicated are in dB SPL ± 5 dB.

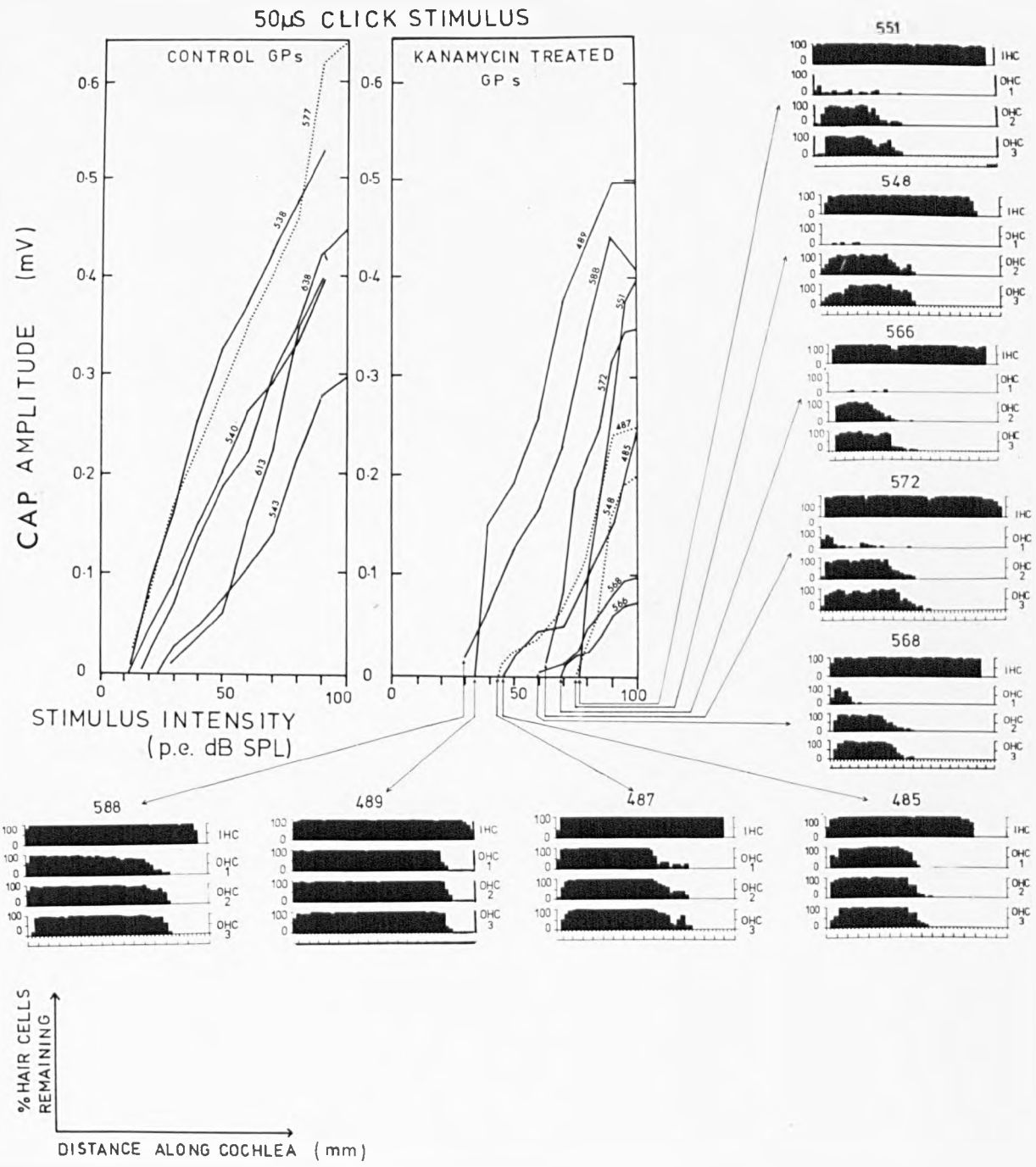


FIGURE 5.18 Click (50 μs) evoked CAP amplitude : intensity functions for normal control GPs (left) and nine kanamycin treated GPs (right). The cochleograms indicate the extent of the cochlear lesion when the amplitude : intensity functions were determined. The amplitude of these functions are plotted absolutely, in mV. The stimulus intensities are given in peak equivalent terms re. a pure tone at 10 kHz.

to a region of the cochlea with total OHC loss. Further examples are shown in figures 5.15-5.17. The latter figure shows that all these functions have similarly elevated thresholds (40-50 dB) and reduced maximum amplitudes. In many cases, there is a tendency for the function to have a plateau at high intensities of stimulation. Because the maximum amplitude of these functions, in cochleas with similar lesions, is less variable than for click stimulation, the slope of the function is less variable (as a comparison of figures 5.17 & 5.18 illustrates). The steepness of these slopes typically approach the slope steepness of normal functions at similar stimulus intensities (c. 5 μV/dB).

The amplitude : intensity functions are discussed in sections 8.2 & 8.3.

The latency : intensity functions (click stimulus) shown in figures 5.15 and 5.16 for severely damaged cochleas are typical for the pattern of hair cell lesion shown (i.e. extensive basal OHC loss). Near threshold, the response has a long latency of 2 ms which rapidly shortens with increasing intensity, and reaches a 'normal' value (approximately 1 ms) at maximum stimulus intensity.

Figure 5.19 shows the result of an experiment during which an acute deterioration of cochlear function occurred caused by cochlear hypoxia. The CAP audiogram, and CAP amplitude and latency functions were measured before (dashed curves) and after (continuous curves) the deterioration. In this case the maximum CAP amplitude changed little; the threshold was elevated by 40 dB. The latency functions show that both at threshold, and at maximum stimulus intensities, the CAP latencies were similar before and after cochlear hypoxia.

These latency : intensity functions are discussed in section 8.4.

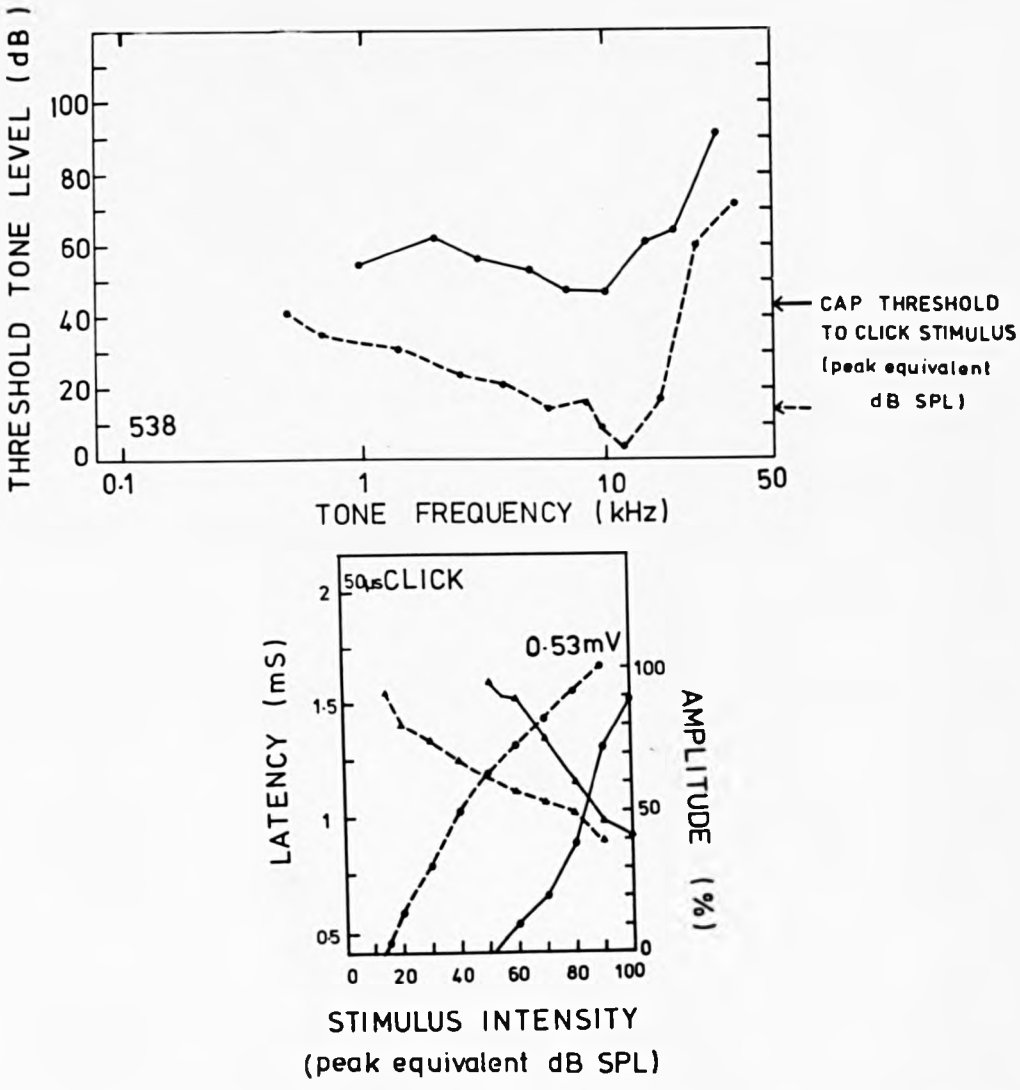


FIGURE 5.19 CAP audiograms, and click evoked CAP amplitude and latency: intensity functions, before (dashed curves) and after (continuous curves) acute cochlear hypoxia. The amplitude of the CAP is expressed as a percentage of its response to maximum stimulation. Stimulus intensity is given in peak equivalent terms re.pure tone at 10 kHz.

CHAPTER 6.

DISCUSSION : HAIR CELL DEGENERATION.

6.1 THE PATTERNS OF HAIR CELL DEGENERATION FOUND IN THE PRESENT STUDY.

6.2 CONSIDERATIONS ON THE QUESTION OF WHETHER HAIR CELLS SURVIVING KANAMYCIN ARE NORMAL.

6.3 EXTENSIVE IHC LOSS FOUND IN THE LONG TERM AFTER KANAMYCIN TREATMENT: POSSIBLE CAUSE AND IMPLICATIONS.

6.4 THE RESISTANCE OF ALBINO GUINEA PIGS TO THE OTOTOXIC EFFECTS OF KANAMYCIN.

6.5 THE ACCURACY OF THE COCHLEAR FREQUENCY MAP USED IN THE PRESENT STUDY.

6.1 THE PATTERNS OF HAIR CELL DEGENERATION FOUND IN THE PRESENT STUDY.

The patterns of hair cell degeneration found in the present study compared well with previous studies of kanamycin damage in GP cochleas. These previous studies included those in which the hair cell degeneration for the whole cochlear length was mapped out in detail (DALLOS & WANG, 1974; YLIKOSKI, 1974) as well as others in which only restricted regions of the cochlea were sampled (e.g. HAWKINS, 1959; WARD & FERNANDEZ, 1961; HAWKINS & ENGSTRÖM, 1964; KOHONEN, 1965; ENGSTRÖM et al. 1966; LUNDQUIST & WERSÄLL, 1966; GONZALEZ et al. 1972; HARSPUR & D'ARCY, 1975) or in which less detailed whole cochleograms were made (ROMAHN & BOERGER, 1977).

The present study has thus confirmed many of the salient features of aminoglycoside induced hair cell degeneration e.g. OHC vulnerability at the basal region of the cochlea; IHC degeneration sometimes starting from the apical region; the symmetry between the patterns of degeneration in the two ears, and the great variation in susceptibility to kanamycin between individual animals.

6.2 CONSIDERATIONS ON THE QUESTION OF WHETHER HAIR CELLS SURVIVING KANAMYCIN ARE NORMAL.

Some of the conclusions drawn from the results of this study concerning the effect of total OHC loss on cochlear response properties (see discussion chapter 7), depend on the validity of the assumption that the IHCs in regions of total OHC loss are themselves unaffected by the kanamycin - as suggested by their appearance under light microscopy. From the present data, no categorical statement can be made on this point, however the following considerations support the assumption.

Firstly, electron microscopic studies on hair cells remaining in GPs after similar regimes of kanamycin administration and recovery period, show no gross structural abnormalities (e.g YLIKOSKI, 1974; see section 1.4d for full review). Secondly, the present study has shown that the responses of cochlear fibres originating from regions of kanamycin treated cochleas where all hair cells remain, are similar in all respects to the responses obtained from normal animals despite, presumably, a sub-critical exposure of the hair cells to kanamycin (compare data indicated by filled circles and filled square symbols in figures 4.7, 4.24, 4.25, 4.26). Thirdly, the close correlation between normal and abnormal cochlear fibre responses and the transition between the presence and absence of OHCs is compelling. There is no reason to expect

an identical locus of IHC damage, particularly in the absence if any morphological correlate of that transition in the IHCs.

These arguments are, of course, not conclusive. The possibility that IHCs remaining after OHC loss are functionally abnormal, because of previous exposure to kanamycin, has to be borne in mind.

6.3 POSSIBLE CAUSE, AND IMPLICATIONS OF THE EXTENSIVE IHC LOSS FOUND IN THE LONG TERM AFTER KANAMYCIN TREATMENT.

The present investigation has found that cochleas poisoned with kanamycin had, in the long term, extensive IHC degeneration as well as OHC loss (section 3.3). Although this IHC loss has been found in other studies, little attention has been drawn to it. ARAN & DARROUZET (1975) using the same kanamycin dosage regime in the GP (400 mg/kg/day for 8 days) found, after many months, both inner and outer hair cell loss at the base of the cochleas.

Another example of this finding came from the work of KIANG and his coworkers. The study of KIANG et al. (1970) was based on eleven kanamycin treated cats, four of which were sacrificed 19-35 days after the final kanamycin injection, and seven of which were sacrificed 135-168 days after treatment. In a later study by KIANG et al (1976), cochlear pathology was investigated in cats 4-5½ years after kanamycin intoxication. Figure (6.1) shows the cochleograms from a sample of the kanamycin treated cats from the two above studies. Cochleograms A & B are from animals sacrificed soon after treatment (19 & 35 days respectively) and show extensive regions of OHC loss for large parts of which the corresponding IHCs are intact. Cochleograms C - F are from the cats sacrificed 135-168 days after treatment, showing more IHC loss than A & B. Cochleogram G (different format) indicates that 4 years after kanamycin, there was no extensive region of OHC loss in which IHCs remained intact. It can also be noted from cochleogram G (graph NF) that extensive neural degeneration has occurred, probably as a consequence of the IHC loss (SPOENDLIN 1973).

In the kanamycin treated macaque monkey, STEBBINS et al. (1969). noted extensive IHC & OHC degeneration after 211 days of continuous kanamycin treatment.

YLIKOSKI (1974), investigating neural degeneration in GP after administration of a variety of aminoglycosides, demonstrated that with long survival times after dosage there was considerable neural degeneration. This is indirect evidence for IHC degeneration because such neural degeneration only occurs after IHC loss (SPOENDLIN, 1973).

In contrast to the results of the present study, and the others cited above, DALLOS (personal comm. 1977) using the same dosage regime as the present study, could not find any evidence of long term IHC loss. The reason for this discrepancy is unclear.

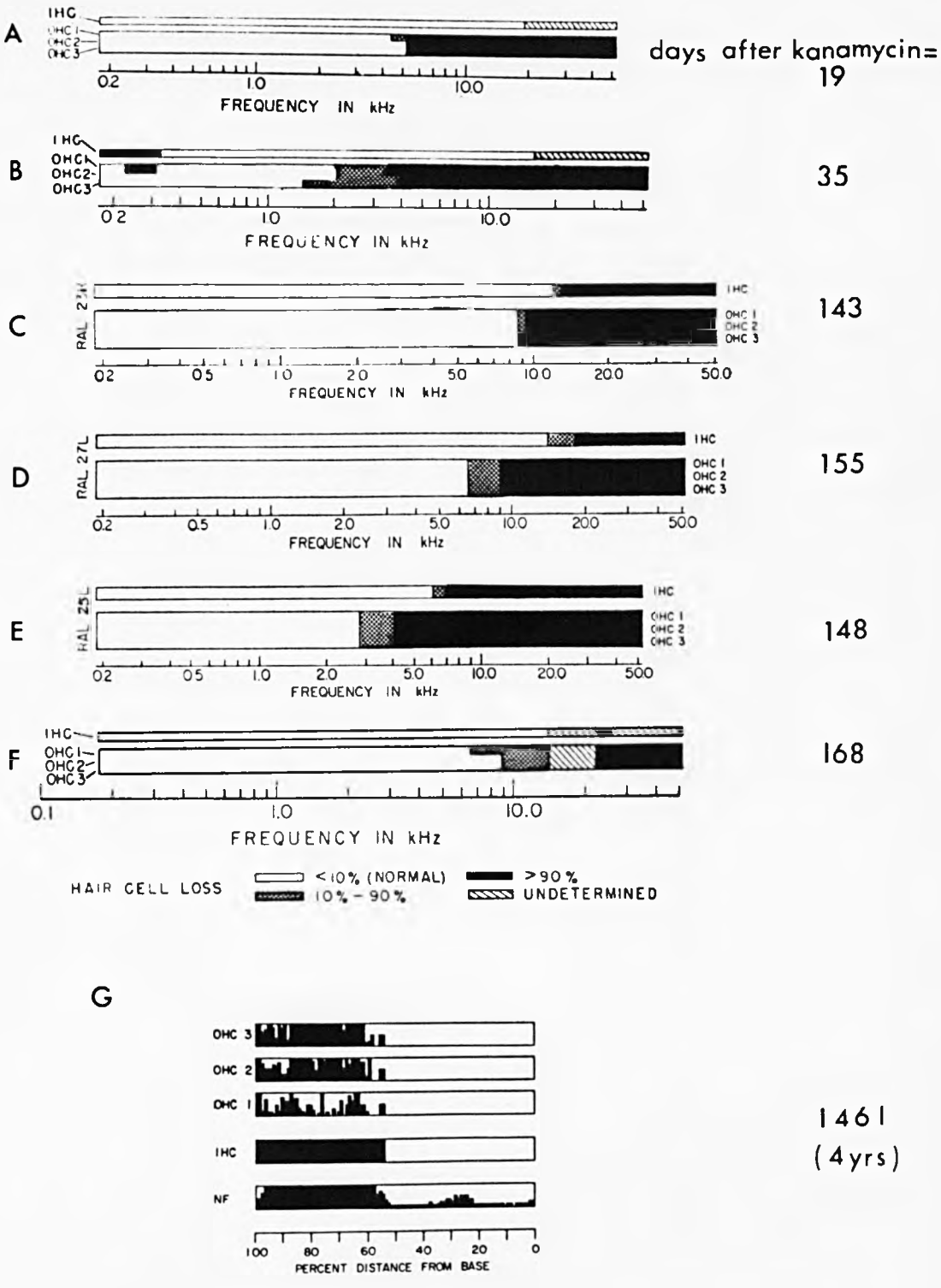


Figure 6.1 (From KIANG et al. 1970; KIANG et al. 1976). Cochleograms of kanamycin treated cats at various times after kanamycin treatment (19 days - 4 years), to illustrate the long term degeneration of IHCs after OHC loss.

A possible cause of long term IHC loss found in this and the other studies mentioned, may have been that during kanamycin poisoning, IHCs were affected as well as the OHCs, but their ultimate deterioration was delayed or had a very slow time course and only manifested itself in the long term. If this were so, then it may cast doubt on the assumption that IHCs are functionally normal after kanamycin intoxication. However, certain aspects of the IHC degeneration argue against the idea that the IHCs were directly affected by kanamycin.

Firstly, from the known sequence of kanamycin induced hair cell degeneration (see section 3.1) it appears that when damage to the IHCs occurs, caused directly by kanamycin, it is manifest by IHC loss at the apex rather than at the base of the cochlea[1] (e.g. KOHONEN, 1965; DALLOS, 1973). This being so, even long term IHC damage, if it was caused directly by kanamycin poisoning, would be expected to damage the apical IHCs first. Clearly this is not the case, as figure 3.6 illustrates, thus arguing against the idea of kanamycin being directly responsible for long term IHC loss.

Secondly, given that all rows of hair cells have different susceptibility to, or different exposure to the ototoxic effects of kanamycin, it is difficult to see why the long term IHC degeneration should always extend up to the region where OHC remain even if there is only a sparse residual population (e.g. 8-11 mm from the basal end of cochlea 545 in figure 3.6). Indeed, it is tempting to suggest that the IHCs are dependent on the presence of some OHCs for their maintained existence. Why this should be can only speculated on. Perhaps a lack of stimulation of the IHCs causes their eventual degeneration; it is clear from the results of the present study that the threshold of response of IHCs in regions of OHC loss is some 40-60 dB above normal. (Implicit in this speculation, if correct, is that the IHCs in intact cochlear regions are stimulated directly or indirectly at low threshold.)

It is clearly essential, before further speculation, to establish with more certainty that the long term IHC degeneration is not the direct result of kanamycin poisoning. One test would be to use ototoxic agents other than kanamycin or related aminoglycosides to produce extensive OHC loss (e.g. ethacrynic acid (MAIZ et al. 1969); atoxyl (sodium arsanilicum, AMJKC. 1976); noise over-exposure), and to then look for a similar pattern of long term IHC loss.

---

[1] Compared with other studies, IHC degeneration at the apex has been less obvious in the results of the present study occurring only in cochleas with very severe hair cell loss - see figure 3.1, cochleograms Q, R, & S.

6.4 THE RESISTANCE OF ALBINO GUINEA PIGS TO THE OTOTOXIC EFFECTS OF KANAMYCIN.

This study has demonstrated (section 3.2) that albino GPs are much more resistant to the ototoxic effects of kanamycin than pigmented animals. Others have also suspected this to the case (DALLOS, 1975 communication to E.F. EVANS; LENG, 1977 personal communication) and this finding raises some interesting questions as to why pigmented GPs are more susceptible. One possible clue could be that the pigmented GPs have melanocytes in their stria vascularis (incidently making cochlear turns easily recognisable and thus aiding cochlear dissection), and that the pigment melanine has an affinity for ototoxic drugs (in vitro) (LINQUIST, 1973). According to LA FERRIERE et al. (1975), melanocytes may have a metabolic or secretory importance in the well vascularized regions of the labyrinth to which they are confined. It is interesting in this context (although not strictly relevant) to mention the recent findings of HOOD, POOLE & FREEDMAN (1976) on the correlation of eye colour with susceptibility to temporary threshold shift, a finding which as they point out could also be connected with the melanine concentration in the stria vascularis. There seems to be a direct correlation between the concentration of melanine in the iris and in the stria vascularis (BONACCORSI, 1965).

6.5 THE ACCURACY OF THE COCHLEAR FREQUENCY MAP USED IN THE PRESENT STUDY.

In the present study, an accurate cochlear frequency map was essential before any useful comparison could be made between the responses of cochlear fibres and the pattern of hair cell degeneration in the cochlear region of origin of those responses. The importance of such a frequency map was emphasized by the study of KIANG et al. (1970). These authors admitted some difficulty in correlating, with certainty, changes in PTCs with cochlear regions of OHC loss because a precise cochlear frequency map was not then available for the cat (KIANG et al. 1976). The accuracy of the cochlear frequency map (of WILSON & JOHNSTONE, 1972) used in the present study has been confirmed by a number of subsequent studies. ROBERTSON & MANLEY (1974) and JOHNSTONE (1977) obtained, in their GP spiral ganglion cell recordings, relatively accurate frequency maps (at least for CFs below 20 kHz) which are consistent with the map of WILSON & JOHNSTONE. A GP frequency map by YLIKOSKI (1974) based on the comparison of abrupt behavioural threshold shifts with abrupt changes in hair cell degeneration patterns is also similar to the mapping used in the present study. Figure 6.2 shows the results of the studies described above compared with the frequency map used in the present study.

Figure 6.3 is a graph similar to that produced by YLIKOSKI (1974) (described above) but instead of plotting discontinuities in the behavioural

audiogram, changes in the CAP audiogram from the results of the present study are compared with regions of abrupt changes in the hair cell degeneration pattern. For example, from the data of figure 5.2 the region of transition between very few OHCs and an almost full complement of hair cells, approximately 5-7.5 mm from the base, is correlated with the steep slope of the CAP audiogram between 5 & 8 kHz. Another example can be seen in figure 5.8, in which the location along the cochlea at the point of total IHC loss is correlated with the high frequency cut-off slope of the CAP audiogram. The diagonal line in figure 6.3 represents the frequency map used in this study (from WILSON & JOHNSTONE, 1972). There is a reasonable agreement between the two sets of data.

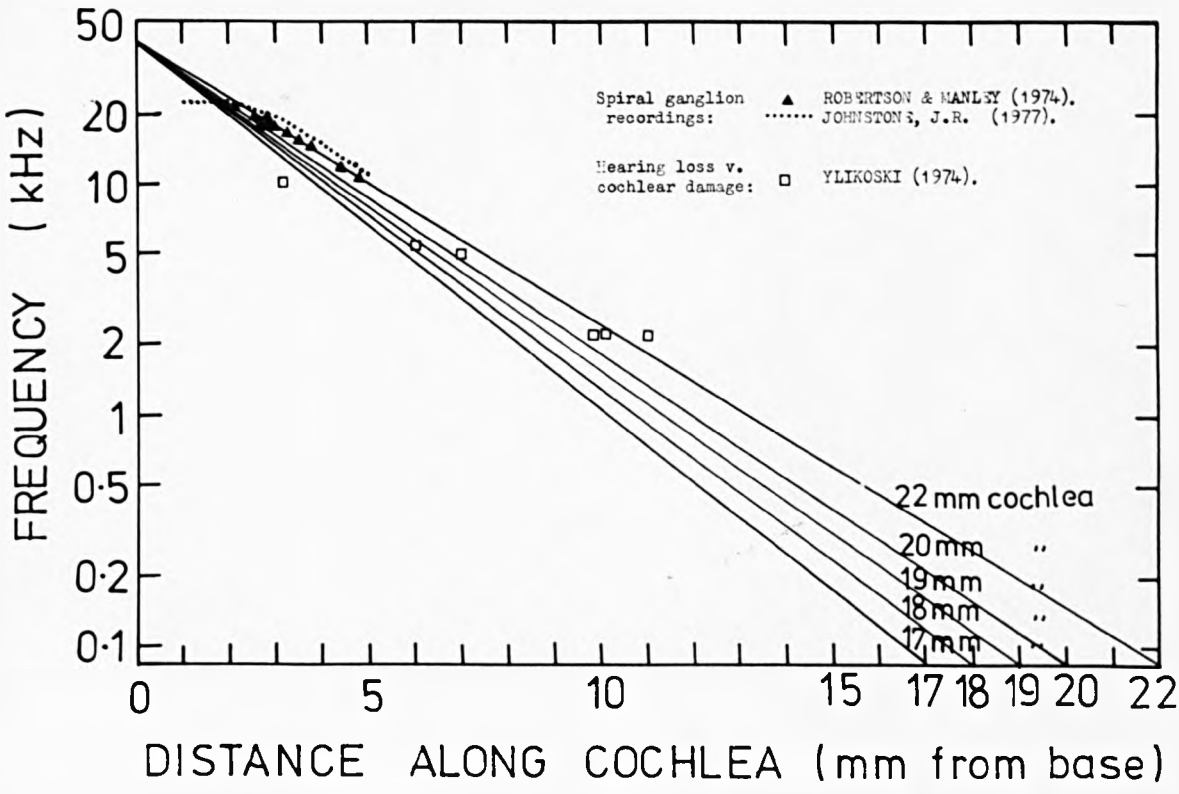


Figure 6.2 The continuous lines show GP cochlear frequency maps used in this study for different cochlear lengths between 17-22mm. These maps are based on that of WILSON & JOHNSTONE, 1972. For comparison are shown some more recent frequency vs. position measurements (see insert for references).

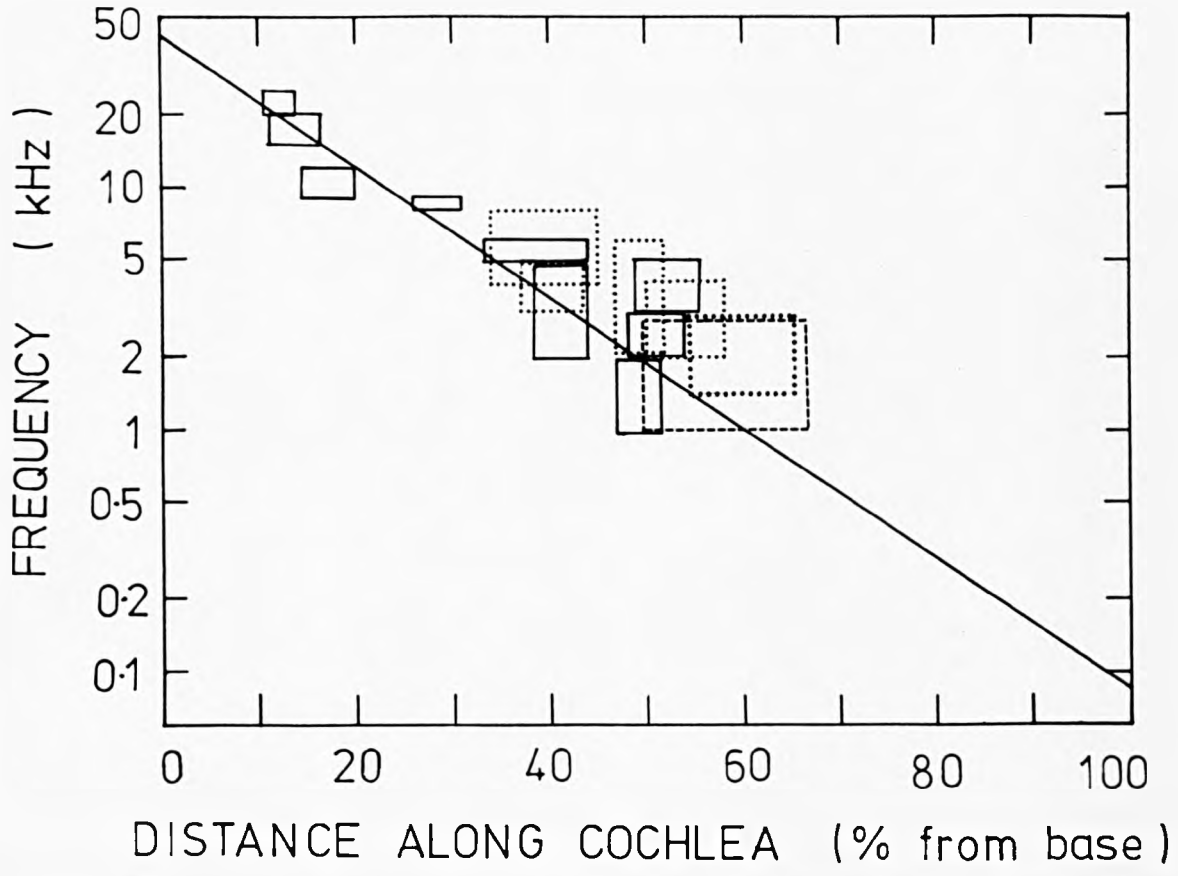


Figure 6.3 GP cochlear frequency map based on the correlation of abrupt irregularities in the pattern of hair cell damage in kanamycin treated cochleas, with associated changes in the CAP audiogram. The horizontal axis of each rectangle represents the region of the cochlea over which an abrupt irregularity in the pattern of kanamycin produced hair cell damage occurred. The vertical axis is the frequency range over which the CAP audiogram showed a discontinuity obviously associated with the hair cell pattern change. The diagonal line is the frequency map used in the present study (WILSON & JOHNSTONE, 1972).

CHAPTER 7.

DISCUSSION: COCHLEAR FIBRE STUDIES.

7.1a RATES OF SPONTANEOUS ACTIVITY IN FIBRES FROM CONTROL ANIMALS, INCLUDING FIBRES FROM COCHLEAS WITH ACUTE PATHOLOGY.

7.1b THE EFFECTS OF OHC LOSS ON SPONTANEOUS ACTIVITY.

7.1c THE RELATIONSHIP BETWEEN NORMAL COCHLEAR FIBRE TUNING OR MINIMUM THRESHOLDS AND SPONTANEOUS ACTIVITY.

7.2a THE MINIMUM THRESHOLDS OF NORMAL COCHLEAR FIBRES.

7.2b THE TUNING PROPERTIES OF NORMAL COCHLEAR FIBRES.

7.3 THE EFFECTS OF OHC LOSS ON THE THRESHOLD AND TUNING PROPERTIES OF COCHLEAR FIBRES.

7.3a MINIMUM THRESHOLDS OF COCHLEAR FIBRES FROM AREAS OF OHC LOSS.

7.3b TUNING PROPERTIES OF COCHLEAR FIBRES FROM AREAS OF OHC LOSS.

7.4 THE RELATIONSHIP BETWEEN COCHLEAR FIBRE TUNING AND THE ELEVATION OF MINIMUM THRESHOLD.

7.5 INNER AND OUTER HAIR CELL INTERACTION.

7.6 AN ANIMAL MODEL OF SENSORINEURAL HEARING LOSS OF COCHLEAR ORIGIN.

7.6a THE ANALOGY BETWEEN GP COCHLEAS WITH HAIR CELL LOSS AND PATHOLOGICAL HUMAN COCHLEAS.

7.6b THE RELATIONSHIP BETWEEN NORMAL PSYCHOPHYSICAL AND NEURAL FREQUENCY SELECTIVITY.

7.6c FREQUENCY SELECTIVITY IN COCHLEAR PATHOLOGY.

7.6d OTHER CHARACTERISTICS OF HEARING LOSS OF COCHLEAR ORIGIN WHICH MAY HAVE NEURAL CORRELATES AT THE COCHLEAR LEVEL.

7.1a RATES OF SPONTANEOUS ACTIVITY IN FIBRES FROM CONTROL ANIMALS, INCLUDING FIBRES FROM COCHLEAS WITH ACUTE PATHOLOGY.

The distribution of mean rates of spontaneous activity, for normal cochlear fibres (see figure 4.3) was not found to differ greatly from previous studies in GP (EVANS, 1972; MANLEY & ROBERTSON, 1976: spiral ganglion cells) or in cat (KIANG et al. 1965).

Previous studies have shown that a high proportion of fibres with abnormally elevated thresholds have low or zero rates of spontaneous discharge. Thus EVANS (1972) reported that 50% of fibres with high minimum thresholds had near zero spontaneous discharge rates of activity. Similarly, MANLEY & ROBERTSON (1976) found that over half the fibres with minimum thresholds above 70 dB SPL had spontaneous discharge rates below 20/s. In both of these cases, the elevations in minimum thresholds were the result of cochlear hypoxia or mechanical damage. In the present study, 30% of fibres recorded under known conditions of cochlear hypoxia had zero rates of spontaneous discharge.

7.1b THE EFFECTS OF OHC LOSS ON SPONTANEOUS ACTIVITY.

Kiang et al. (1970) found that 30-50% of fibres from cat cochleas with OHC degeneration caused by kanamycin poisoning, had zero rates of spontaneous discharge. On the other hand, DALLOS et al. (1977) reported that in the chinchilla, the rates of spontaneous discharge in fibres from cochlear regions of OHC loss (due to kanamycin) were no different from those of normal fibres.

The results of the present study (figure 4.3) clearly indicate that 50% of cochlear fibres from regions of total OHC loss had zero or near zero rates of spontaneous discharge (compared with less than 11% in normal cochlear fibres). Many fibres (30%) from cochlear regions of partial OHC loss also had low or zero rates of spontaneous discharge.

It is most reasonable to start with the assumption that because most of the afferent cochlear fibres terminate on the IHCs, that either the IHC synapse or the membrane of the afferent dendrite[1] is the site of origin of spontaneous activity.

It could be argued that the change in the distribution of rates of spontaneous activity which accompanies OHC loss is evidence that the OHC somehow modulate the spontaneous activity in cochlear fibres. On the other hand, because only 50% of fibres from regions of total OHC loss have low or zero rates of spontaneous activity, and the range of rates for the other fibres is normal,

---

[1] see over for foot note.

it could be supposed that some fibres have normal rates of spontaneous activity in the absence of OHCs (i.e. that spontaneous activity in these fibres is independent of the OHCs). Both arguments, on the evidence of the data from this study, remain inconclusive.

The claim of Dallos et al (1977) that, in the chinchilla, there was no change in the distribution of spontaneous activity in cochlear fibres after OHC loss favours the interpretation that the OHCs do not influence the rate of spontaneous activity. If this is the case, then the question is raised as to whether the low rates of spontaneous activity found in the pathological fibres of the present study are the direct result of kanamycin on the IHCs (assuming that the IHCs are responsible for spontaneous activity). It is worth pursuing this line of discussion because there is an important difference between the results of the study by DALLOS et al. (1977) on kanamycin treated chinchilla and the results of the present study in a respect other than the spontaneous rates of activity. More specifically Dallos and his co-workers claimed that the tuning of cochlear fibres did not deteriorate after total OHC loss (see section 7.3b). It could be argued that the low rates of spontaneous activity in many of the cochlear fibres of the present study and the deterioration in the tuning of cochlear fibres after OHC loss are both manifestations of IHC damage caused directly by the kanamycin (and not occurring in Dallos's study, presumably because of the species difference and/or differences in the kanamycin dosage regime).

With regard to the above proposal it is important to see whether the (residual) tuning properties of fibres from cochlear regions of total OHC loss are in any way related to the rates of spontaneous activity of those fibres. In figure 7.1, the spontaneous rate of activity (spikes/s) of cochlear fibres from regions of total OHC loss are plotted against the tuning ($Q_{10\ dB}$) values of the fibres. The linear regression is shown by the dashed line. The correlation co-efficient is very low. There is clearly no relationship. This is evidence against the argument that the deterioration in tuning of cochlear fibres after OHC loss and the low rates of spontaneous activity may be closely correlated and associated with IHC abnormality.

---

1 Although some authors have suggested that the membrane of the afferent dendrite may contribute to spontaneous activity production (KONISHI et al. 1970; TEAS et al. 1970; MANLEY & ROBERTSON, 1976), most authors favour the hair cell synapse as the site of origin of spontaneous activity (e.g. FLOCK, 1971; WALSH et al. 1972; KLINKE & EVANS, 1977). It may be reasonable to assume that the spontaneous activity is associated with a leaky chemical synapse as in other neural systems e.g. motor nerve endings (FATT & KATZ, 1951) or, more closely related, in primary neurones of the lateral line system (FLOCK, 1971; FLOCK & RUSSELL, 1976) and goldfish sacculus (FURUKAWA & ISHII, 1967; FURUKAWA et al. 1972).

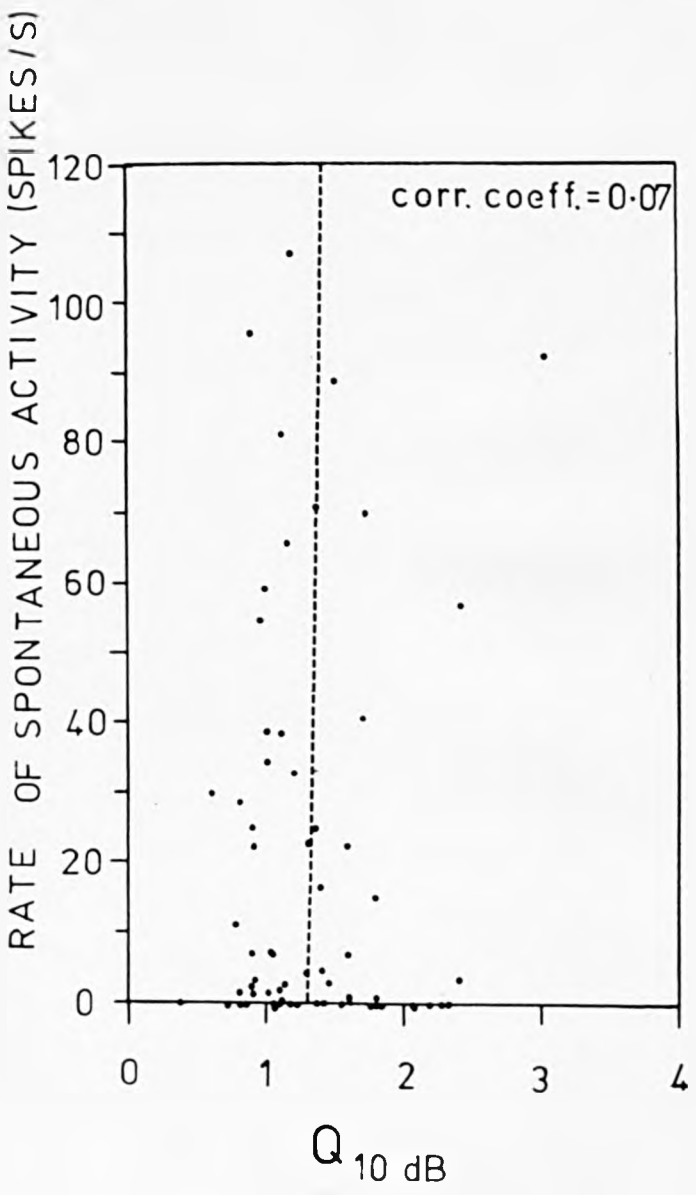


FIGURE 7.1 The rate of spontaneous activity of afferent fibres from cochlear regions of total OHC loss, plotted against the tuning ($Q_{10\ dB}$) of the fibre. The linear regression is shown by the dashed line.

It must be repeated that, from the present data, the cause of the low or zero spontaneous activity cannot be decided upon. The possibility that kanamycin has some direct action on the IHCs (the effect of which persists for many weeks) cannot be ruled out. This question requires testing. It would, for example, be useful to ascertain whether kanamycin administration results in an immediate change in the spontaneous activity of cochlear fibres, or whether changes in the distribution of spontaneous activity develope over a longer time period (e.g. in parallel with the degeneration of OHCs). It would also be informative to produce selective OHC loss with agents other than kanamycin (see page 111, para. 4) to find out whether the low rates of spontaneous activity were perhaps exclusively the result of kanamycin intoxication.

7.1c THE RELATIONSHIP BETWEEN NORMAL COCHLEAR FIBRE TUNING OR MINIMUM THRESHOLDS AND SPONTANEOUS ACTIVITY.

The present study has failed to find any relationship between the spontaneous activity and either the minimum thresholds or the tuning (10 dB bandwidth) of normal cochlear fibres.

In figure 4.5, which shows spontaneous rate of discharge of fibres plotted against their minimum thresholds, the correlation coefficient of the data is very low, and together with the large scatter indicates no strong relationship. Similarly in figure 4.6, which shows spontaneous rate of discharge plotted against 10 dB bandwidth, there is no clear relationship. Although the linear regression (dashed line) could be said to indicate a trend for high spontaneously active fibres to be more sharply tuned, the low correlation coefficient (-0.181) makes such a trend insignificant. It is interesting to note that in contrast to the latter findings, KIANG et al. (1976) have claimed that units with low rates of spontaneous activity tend to be more sharply tuned than those with high rates. Unfortunately they illustrated their finding with a figure (fig.5 in KIANG et al. 1976) which, on close scrutiny, does not support the claim.

Further evidence which suggests that threshold (and tuning) properties are independent of spontaneous activity comes from EVANS who has demonstrated that the spontaneous discharge rate of a cochlear fibre can remain virtually unchanged during changes in threshold and tuning caused by cochlear hypoxia (unpublished observation) and instillation of KCN into the cochlea (EVANS, 1976a experiments of EVANS & KLINKE, 1974).

ROBERTSON (1974) found, in GP spiral ganglion recording, that threshold elevation and changes in tuning caused by drainage of the perilymph occurred "with no alteration of both the mean rate and the interspike interval of the spontaneous activity".

MANLEY & ROBERTSON (1976) have also demonstrated that the minimum threshold of a cochlear fibre can change independently of spontaneous activity during cochlear hypoxia. Thus, these authors noted that after cochlear hypoxia, which caused an elevation of minimum threshold and a reduction in spontaneous discharge, the recovery of spontaneous rate of discharge lagged behind the recovery of normal minimum threshold. They also compared the minimum threshold values of normal cochlear fibres with their rate of spontaneous activity and found no correlation between them. Similarly, in amphibia, CAPRANICA & MOFFAT (1975) failed to find any obvious relationship between the rate of spontaneous activity in auditory nerve fibres (in spade foot toad) and the response properties of those fibres to acoustic stimuli (e.g. minimum thresholds).

7.2a THE MINIMUM THRESHOLDS OF NORMAL COCHLEAR FIBRES.

The distribution of the minimum thresholds of normal cochlear fibres with their CFs found in the present study was similar to that found by EVANS (1972). One interesting feature of both these studies is the discrepancy between the behavioural audiogram (dashed line in figure 4.7 from HEFFNER et al. 1971) and the minimum thresholds at CFs above 15 kHz.[3] The first aspect to this discrepancy is that the behavioural thresholds are lower than those of cochlear fibres particularly at frequencies above 15 kHz. The most obvious explanations for this are: a) as suggested by EVANS (1972), a relatively high criterion of threshold was used in the present experiments; it is extremely likely that over a large population of

---

2 see 'additional remarks' after EVANS, 1974b.

3 The low behavioural thresholds in the GP have recently been confirmed at 32 kHz by WALLOCH & TAYLORSPIKES (1976) using a different behavioural technique (food reward) than that of HEFFNER et al. (conditioned suppression).

cochlear fibres, a lower criterion of threshold is used compared with the threshold detection in the unaveraged response of a single cochlear fibre; b) because of the ability of the animal (in behavioural experiments) to optimize the position of its head in the sound field, there could be a difference between the assumed SPL at the tympanic membrane, and the actual SPL (even after correcting, as far as possible, the free field SPL for the characteristics of the external meatus and pinna).

The second aspect of this discrepancy is the apparent lack of cochlear fibres with CFs above 20-25 kHz, a feature found in the present study[4], in that of EVANS(1972), and more recently by JOHNSTONE (1977). JOHNSTONE recorded from spiral ganglion cells at the extreme basal region of the cochlea, and was unable to record from any fibres with CFs higher than 20-25 kHz. With respect to nerve fibre recordings (EVANS 1972, and the present study), the lack of data from fibres with high CF may have been because they are positioned on the posterior aspect of the cochlear nerve in a restricted surface layer; most electrode tracks in the present study were made through the middle (the widest part) of the nerve and little effort was made to sample areas most likely to contain high CF units.

With regard to JOHNSTONE'S spiral ganglion recordings, MANLEY (1977, comment on JOHNSTONE, 1977) has suggested that the extreme basal end of the cochlea may be extraordinarily susceptible to adverse conditions such as cochlear hypoxia or mechanical overstimulation; the spiral ganglion recordings do require the potentially traumatic procedure of opening and draining of the basal scala tympani[5]. MANLEY'S suggestion could also apply to cochlear nerve fibre recordings where inadvertent restriction of the local blood supply to the cochlea would result in cochlear hypoxia - and render high CF fibres pathological. However, it is clear from CAP audiograms of normal GPs (recorded early in the experimental proceedings and before interference with cochlear blood supply was possible) that there were few animals which produced CAP responses to tone pip stimuli as high as 43 kHz. (See the normal CAP audiograms of figure 5.1 for typical examples.)

---

4 A recent experiment on a kanamycin treated GP (the minimum threshold data of which were not included in figure 4.7) yielded four fibres with CFs around 37 kHz. However, the thresholds of these fibres were pathological and thus it was not easy to define exactly a minimum threshold and therefore CF. See figure 4.23 for examples. (The FTCs are not corrected for the sound system.)

5 Opening and draining of the basal scala tympani can be achieved in GP without a deterioration in the threshold & tuning properties of cochlear fibres (EVANS, 1970b). However the degree of draining is clearly critical (GP: ROBERTSON, 1974; cat: EVANS & WILSON , 1975).

It could be argued that before the CAP audiogram can be confidently taken as a valid measure of high CF cochlear fibre thresholds it must be assumed that the contribution of fibres with high CF is not less than that for other cochlear fibres. It may be the case that there is a considerable decrease in the afferent innervation density from the base of the GP cochlea (as SPOENDLIN, 1973 has shown for the total innervation density for the cat, and as MANLEY, 1977 (comment on JOHNSTONE, 1977) reported for the spiral ganglion cell population in GP), which would reduce the amplitude of the CAP of such fibres and thus,its detectable threshold. However, a total lack of any CAP response to high frequency stimuli would not be expected.

In one experiment on a (kanamycin treated) GP, a CAP audiogram was recorded which extended up to 48 kHz. This animal also yielded recordings from fibres with CFs around 37 kHz[4]. This is circumstantial evidence to support the idea that CAP responses to high frequency stimuli do represent the presence of cochlear fibres responding at those frequencies and furthermore points to the obvious possibility of a variation in the hearing range of individual GPs. In this respect, the correlation of minimum thresholds of cochlear fibres with the behavioural audiogram may be much closer in individual animals.

7.2b THE TUNING PROPERTIES OF NORMAL COCHLEAR FIBRES.

The FTCs of normal cochlear fibres were similar in most respects to those found by EVANS (1972; compare figure 4.9 with 1.4). One discrepancy was that EVANS results indicated that sharpness of tuning was maximal for fibres with CFs around 8 kHz - similar to that of the cat (e.g. EVANS & WILSON, 1971, 1973). In the present study, no such obvious peak in sharpness of tuning was found at 8 kHz. Thus in figure 4.24 in which the $Q_{10\ dB}$ is plotted against the CF of each fibre, the trend for the increase in sharpness of tuning, with increasing CF, is uninterrupted.

In the GP data of EVANS, many fibres with CFs above 10 kHz had $Q_{10\ dB}$ values of less than 6. Low values such as this were found in the present study but only in conditions of known cochlear pathology (small filled circles in figure 4.24). It is possible that, in the present study, the well maintained systemic blood pressure due to the improved anaesthetic technique contributed towards the prevention of acute cochlear hypoxia; EVANS (1972) used urethane or pentobarbitone anaesthesia, under which, GPs are much more prone to cardiovascular depression.

7.3 THE EFFECTS OF OHC LOSS ON THE THRESHOLD AND TUNING PROPERTIES OF COCHLEAR FIBRES.

The first conclusion to be drawn from the data, taken at face value,

is that since cochlear fibres originating in areas of hair cell loss (restricted to OHCs) lose their normal low thresholds and sharply tuned properties, these properties must in some way be dependent upon the functional integrity of the OHCs. This conclusion depends on the critical assumption that the IHCs in the regions of OHC loss are themselves unaffected by the kanamycin as suggested by their appearance to light microscopy. No categorical statement can be made on this point, however, a number of considerations support the assumption. These considerations were discussed in section 6.2.

7.3a MINIMUM THRESHOLDS OF COCHLEAR FIBRES FROM AREAS OF OHC LOSS.

The finding of elevated fibre thresholds in cochlear regions of OHC loss is in agreement with the observations of KIANG and his co-workers (KIANG et al. 1970; KIANG et al. 1976; in cat) and is implicit in the more recent findings in chinchillas by DALLOS et al. (1977). Both studies indicated that the minimum thresholds of cochlear fibres were elevated 40-60 dB after total OHC loss (see e.g. figures 10 & 14 in KIANG et al. 1970). Furthermore these results are entirely consistent with the findings that the behavioural threshold in the chinchilla (RYAN & DALLOS, 1974) and in GP (YLIKOSKI, 1974) were elevated some 40-60 dB for frequencies corresponding to cochlear areas of total OHC loss (in the presence of apparently normal IHCs).

However, ROMAHN (1974; ROMAHN & BOERGER, 1976) did not obtain similar results and claimed that some cochlear fibres from regions of total OHC damage had normal minimum thresholds. These authors have used, as a criterion of hair cell damage, " a disorganization of the hair pattern" in addition to complete hair cell degeneration. This adoption of a criterion of partial hair cell damage was unwise, because histological evaluation cannot reliably indicate the functional integrity of a cell.

In any case, it is not clear from their published cochleograms that they have recorded from fibres innervating cochlear regions of <u>total</u> OHC

damage. Thus, the FTCs illustrated to support their claim (figure 5 in ROMAHN & BOERGER, 1976) were obtained from fibres which, according to their cochleogram, came from near a border dividing a region of '50-90% OHC damage' from a region of 'greater than 90% damage'. Because partial OHC damage is associated with partial and unpredictable degrees of threshold elevation, it is important to establish the region of total OHC loss exactly. The conclusions drawn by ROMAHN & BOERGER would also be more convincing if they had more extensive data from individual animals. Their conclusions should be treated cautiously.

On the question of the effects of OHC loss on threshold, it is concluded that with the exception of the study by ROMANN & BOERGER, to which objections have been made, there is good agreement on the close correlation between total OHC loss and elevations of neural and behavioural thresholds.

7.3b COCHLEAR FIBRE TUNING PROPERTIES OF COCHLEAR FIBRES FROM AREAS OF OHC LOSS.

No normally tuned cochlear fibres were found with CFs corresponding to areas of total OHC loss. The 10 dB bandwidths of the FTCs of these fibres were 5-10 times wider than normal, and the steepness of both their high & low frequency cut-off slopes was considerably decreased (figures 4.25 & 4.26).

These findings of broadly tuned responses in areas of OHC loss are a confirmation of the findings of KIANG et al. (1970) in kanamycin treated cats. However, KIANG and his co-workers could not draw definite conclusions concerning the relationship between OHC loss and the response properties of the fibres because the hair cell lesions in their animals had only restricted regions in which only the OHCs were missing.[6] Thus, KIANG et al. (1976) stated that "although it is tempting to suggest that these broad tuning curves belong to fibres that innervate IHC's in regions where OHCs are missing the lack of a more precise cochlear frequency map precludes such a specific conclusion".

In the present study, there was little difficulty in positively correlating fibres with pathological tuning and threshold properties with regions of total OHC loss because in many cochleas, over a third of their length was such a region (see figures 4.14-4.19).

---

[6] A reason for such restricted regions of selective OHC loss is the long period allowed between kanamycin treatment and the electrophysiological recordings. See sections 3.3 & 6.3.

DALLOS et al. (1977) have recently questioned the finding that cochlear fibres from regions of OHC loss show a deterioration in tuning. They found, in the chinchilla, that 60% of fibres from areas of OHC loss had small, relatively sharply tuned regions as much as 30 dB above minimum threshold on the high frequency cut-off slope of otherwise broadly tuned FTCs. Figure 7.2, from DALLOS et al. (1977), shows two examples of these FTCs. They have measured the $Q_{10\ dB}$ values of these small tips (by extrapolation for many of them, as they often extended for only a few dB), and claimed that these values indicated the FTCs to be effectively as sharply tuned as normal cochlear fibres in the chinchilla. Incidentally, normal chinchilla cochlear fibres are, according to the control data of DALLOS et al, less sharply tuned than those of the GP (of comparable CF). Thus, for CFs around 10 kHz, the average $Q_{10\ dB}$ value of normal chinchilla cochlear fibres was found to be 5 compared with 8 i.. GP.

In the present study, these sharply tuned tips were not a feature found regularly. However, there were often, as some of figures 4.10-4.21 indicate, small prominences or tips at the minimum threshold of broadly tuned FTCs. The $Q_{10\ dB}$ values of these tips, measured by extrapolation, indicated them to be much less sharply tuned than normal GP fibres. Figure 7.3 shows the comparison between these 'tip' $Q_{10\ dB}$ values, (open squares) and values of normal cochlear fibres (large filled circles).

ZWISLOCKI (personal communication) in his studies with SOKOLICH (e.g. ZWISLOCKI & SOKOLICH 1974) on kanamycin treated gerbils has also not found these sharply tuned segments on the high frequency cut-off slopes of pathological FTCs. However, he did not doubt their existence and claimed that in his own studies "they had not been specifically looked for". Indeed, the possibility exists that the tuning curves found by DALLOS have not been found in the present study because of the manual FTC plotting methods used. DALLOS (in discussion on DALLOS et al. 1977) suggested that the broadly tuned FTCs of this present study could represent only FTC tails, and that sharply tuned tips at higher frequencies had been missed. As pointed out by EVANS (in discussion of DALLOS et al. 1977) this was not possible. However, the FTCs found by DALLOS could be thought of as broadly tuned, with a sharp notch in the HF cut-off slope; such a notch could be missed. For this reason, automatic and semi-automatic FTC plotting procedures were used to measure the FTC in detail. Figures 4.22 & 4.23 show representative samples of the results from some of these experiments.

In one cochlear fibre, an FTC was found which resembled the findings of DALLOS et al. (1977). This is shown in figure 7.4. In this animal the FTCs were measured by a semi-automatic procedure in which the frequency of a tone (gated) was slowly changed (0.5 octave/minute) while its intensity was

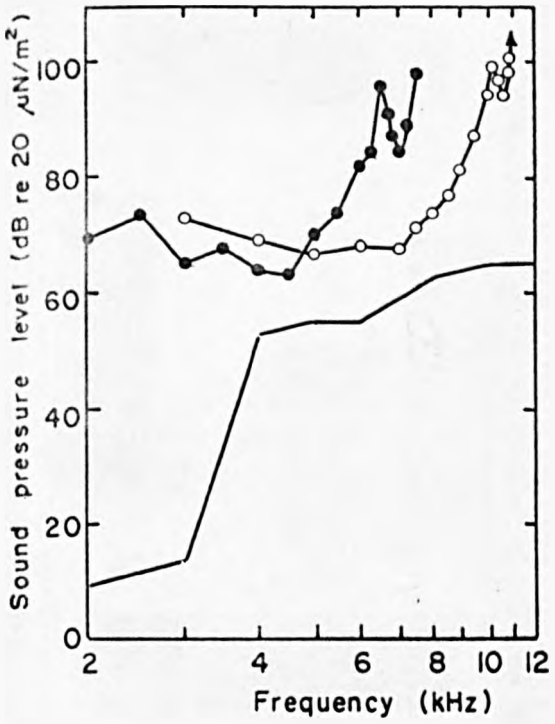


Figure 7.2 (From Dallos et al. 1977). Two cochlear fibre FTCs from a kanamycin treated chinchilla. The fibres are assumed to emanate from an area of the cochlea with total OHC loss.

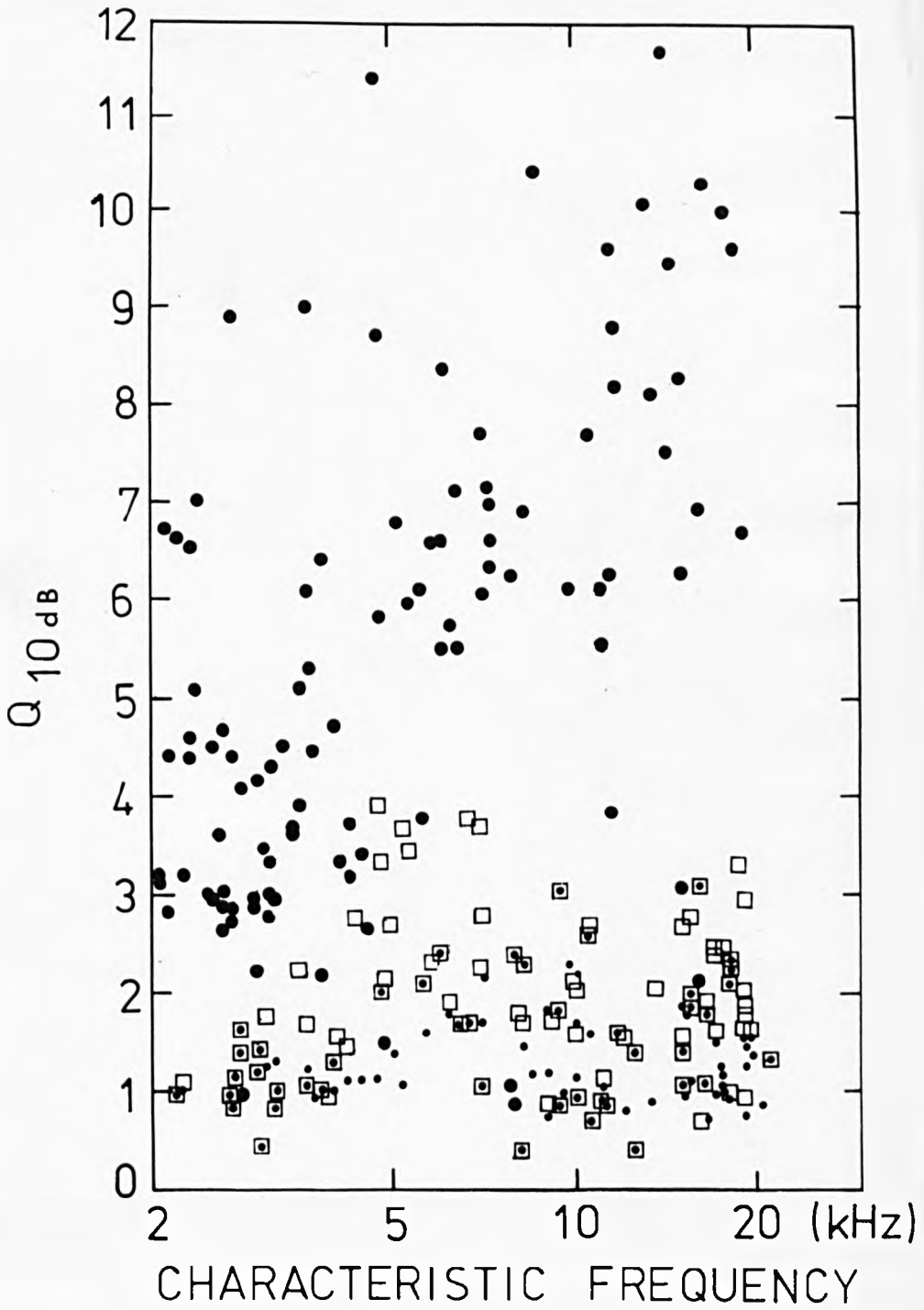


Figure 7.3 $Q_{10\ dB}$ values for normal GP cochlear fibres (large filled circles) compared with values of fibres from regions of total OHCloss (small dots and open squares), plotted against the CF of each fibre. The small dots are $Q_{10\ dB}$ values computed directly from FTC data. The open squares represent values measured by extrapolation of any FTC 'tips'.

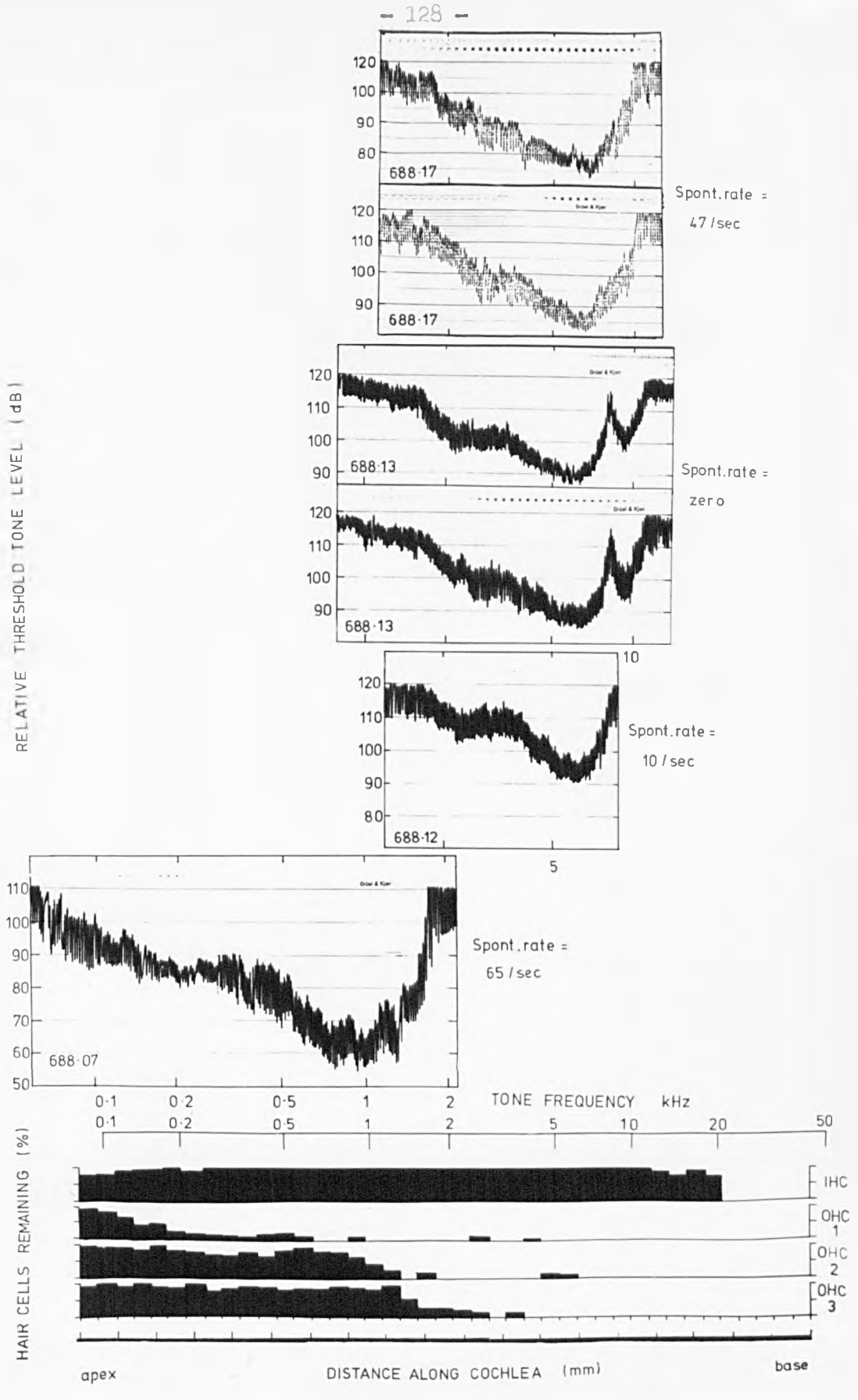


Figure 7.4 Frequency threshold curves of four cochlear fibres from a kanamycin treated GP measured by a semi-automatic procedure in which the frequency of a tone (gated) was slowly changed (0.5 octave/min) while its intensity was repeatedly and by manual adjustment increased to find threshold, and then reduced well below threshold. The stimulus levels are given in relative terms of the electrical input to the condenser earphone, and they represent the approximate intensity (±6 dB SPL) at the tympanic membrane. The cochleogram below shows the percentage of hair cells remaining in the cochlea at the time of the physiological recording.

repeatedly and by manual adjustment increased to find threshold and then reduced well below threshold. Unit 688.13 had an FTC with a notch on the high frequency side of minimum threshold. This feature could be consistently repeated (as shown in the figure). Other fibres in this GP did not show such FTCs (e.g. units 12 & 17). The $Q_{10\ dB}$ value of the tip segment of the FTC (unit 13) was only 3.6, thus it was not as sharply tuned as normal cochlear fibres of similar CF (with average $Q_{10\ dB}$ of 7).

In the GP, the occurrence of these tuning curves is much less than that found in the chinchilla. Furthermore, from the present data, there is no reason to believe that any residual tip to FTCs of cochlear fibres from regions of OHC loss even approaches the sharp tuning of normal cochlear fibres.

DALLOS et al (1977) have measured 'behavioural tuning curves' (tone on tone masking curves; DALLOS & RYAN, 1975) in kanamycin treated chinchillas at frequencies corresponding to total OHC loss. Such behavioural tuning curves were found to be sharply tuned. They suggested that the sharply tuned "tips" of the cochlear fibres FTCs which they find after total OHC are responsible for these sharp behavioural tuning curves. This interpretation is questionable - it is not clear how such sharp behavioural tuning near hearing threshold could be achieved on the basis of sharply tuned regions up to 30 dB above the minimum threshold of the FTC and above the level at which the psychophysical measurements were obtained. (Compare the sharp behavioural selectivity at threshold (70 dB SPL) in figure 1 of DALLOS et al. (1977) with the FTCs in their figure 2.)

Bearing in mind the assumptions which must be made concerning the state of the IHCs, it is concluded that the sharp tuning properties of normal cochlear fibres are dependent in some way on the integrity of the OHCs.

7.4 THE RELATIONSHIP BETWEEN COCHLEAR FIBRE TUNING AND ELEVATION OF MINIMUM THRESHOLD.

Studies on the effects of acute cochlear pathology (EVANS & KLINKE 1974; EVANS 1974c; MANLEY & ROBERTSON 1974; EVANS 1975b,c) raised the question of the relationship between the bandwidth of a fibre's FTC and its elevation of minimum threshold. This question receives a more conclusive answer with the more numerous and systematic data of the present study in chronic cochlear pathology. As figure 4.23 indicates, for fibres with CFs above about 2 kHz, the relationship between threshold elevation (in dB) of a fibre and the 10 dB bandwidth of its FTC was non-linear. The bandwidth changed little until some 30-40 dB of threshold elevation occurred. Similar changes in tuning with threshold shift were demonstrated in cat as a result of furosemide and cyanide intoxication (EVANS & KLINKE, 1974; EVANS, 1974c). Figure 7.5 , taken from EVANS 1974, shows the effect of cyanide on the FTC of a cat cochlear fibre. Only when the minimum threshold had been raised by 30-40 dB (curve A to curve C) did the bandwidth become very much broadened. Figure 4.30 provides an analogous qualitative description of changes in the FTC due to

(b) Cyanide

KCN, in concentrations of about 10^{-3}M was instilled directly through the round window in cats. The changes shown in Fig. 3 were found in fibres with CFs above 7kHz. Loss of the low threshold sharply tuned segment (from A to C) and near total recovery (C to F) could be obtained, again without substantial effects on the cochlear microphonic.

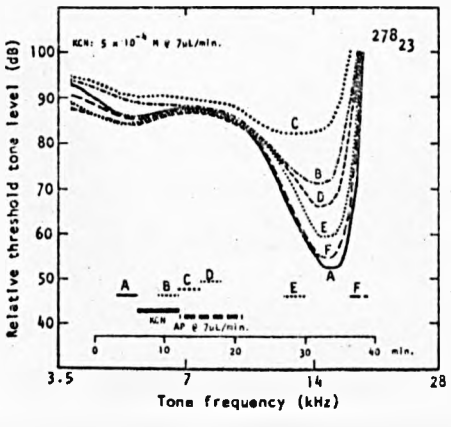


Figure 7.5 (From EVANS 1974c). The effect of KCN, instilled directly into the cat cochlea, on the FTC of a cochlear fibre. The changes shown are typical for fibres with CFs above 7 kHz. The inset shows the time course of the reversible changes.

OHC loss (note that for clarity, the FTCs of this figure are not directly superimposed).

It has been proposed (e.g. EVANS 1974c) that in acute intoxication, it could be the OHCs which are most deleteriously influenced.[7] The similarity between the threshold and tuning changes resulting from acute intoxication with furosemide and cyanide, and the results of the present study, in which the anatomical lesion is clearly at the OHC level, support the proposition.

The changes in FTC shape with elevation of minimum threshold (figure 4.30) gives a clear impression of a sharply tuned low threshold segment being reduced with increasing OHC loss (accompanied by relatively small changes in bandwidth) and being completely eroded when total OHC loss occurs. These observations lend support to the hypothesis (proposed by EVANS e.g. 1974c) that the FTC is made up of two independent segments: a low threshold and sharply tuned part dependent on the OHCs, and a less vulnerable high threshold segment giving rise to the tail of normal FTCs. The results of this study indicate that the IHCs alone give rise to a broadly tuned, high threshold FTC which could constitute the high threshold segment of a normal FTC. A direct comparison between the high threshold segment of a normal FTC with the same fibres FTC after OHC loss is obviously not possible. However, a comparison of typical examples of normal FTCs and FTCs of fibres (with similar CFs) from regions of total OHC loss (IHC FTCs) is shown in figure 7.6. Four normal FTCs and four 'IHC FTCs' are shown from each of three frequency regions (20 kHz, 8 kHz and 2 kHz). For fibres with CFs around 8 kHz the threshold of the tail of normal fibres is, on average, significantly lower than the threshold of the corresponding IHC FTC. For fibres with CFs near 20 kHz, there is less discrepancy. In high CF fibres (>5 kHz) the distinction between the sharp tip segment and the high threshold tail segment is easily made. For low CF fibres, analogous segments can be recognised, although the difference between them in terms of both threshold and tuning is much less obvious. It is also interesting to note that if the pairs of normal and IHC FTCs in figure 7.6 were aligned according to the limit of their high frequency cut-off slopes rather than their CFs, then for high CF fibres, the

---

[7] It has been suggested (SPOENDLIN 1974, additional remarks on EVANS 1974c) that during cochlear hypoxia, the changes in cochlear fibre thresholds and tuning could be the result of damage to the inner radial fibre dendrites, on which small swellings can be seen after 2-3 minutes of anoxia (SPOENDLIN 1970). It can be argued, however, that damage to the dendrite is most likely to have a non specific influence on the response properties of the fibre (e.g. a general depression of spike generation) rather than a specific effect on the low threshold, sharply tuned segment of the FTC. On the other hand it may be significant that the relationship between threshold and tuning during acute cochlear hypoxia seems to differ from the non linear relationship found during the acute cyanide and furosemide intoxication. The changes in tuning tend to go hand in hand with the minimum threshold elevation. (EVANS & WILSON, 1973; ROBERTSON & MANLEY 1974).

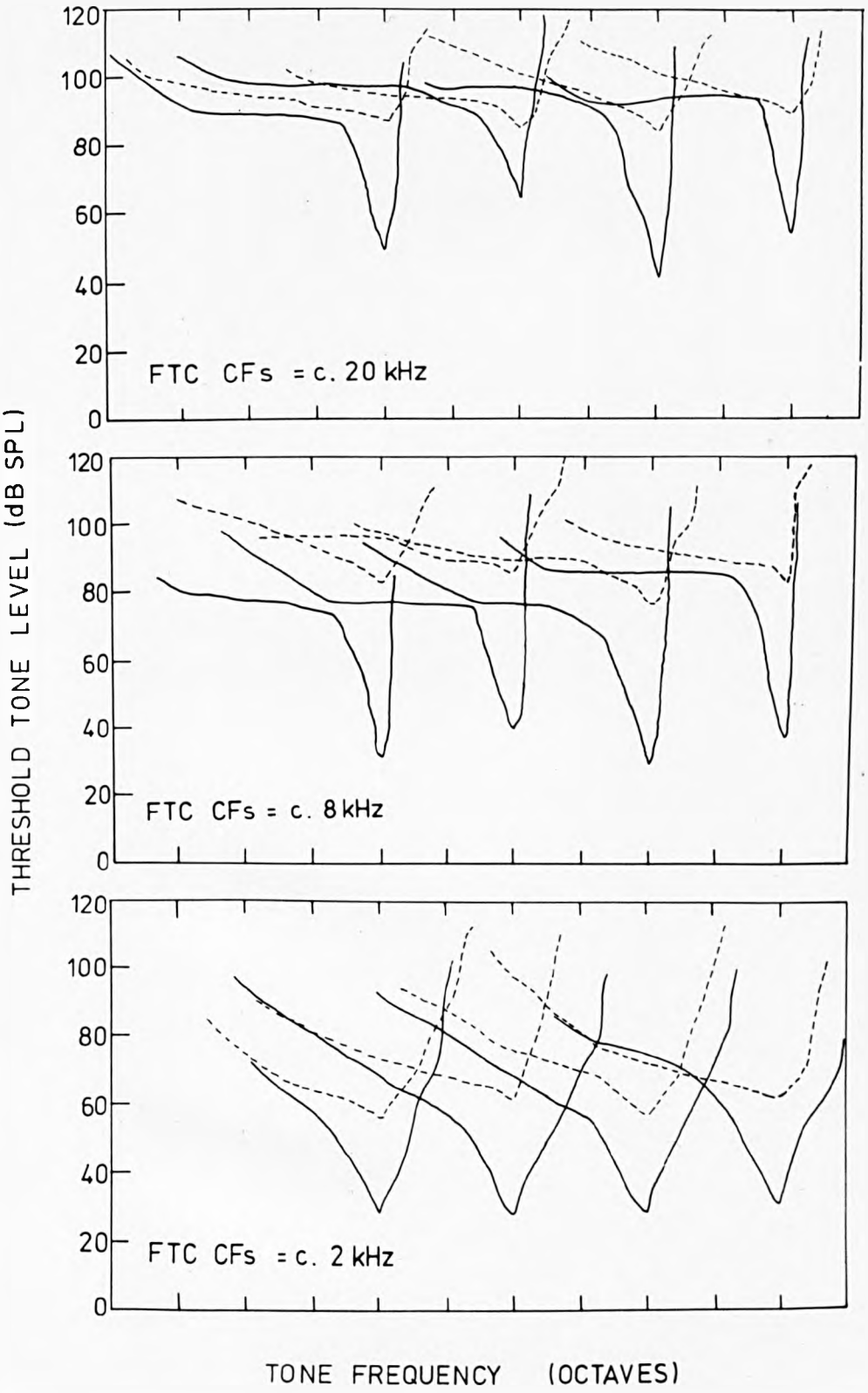


Figure 7.6 A typical sample of FTCs of GP cochlear fibres from normal cochlear regions (continuous curves) compared with FTCs of fibres innervating areas of total OHC loss (dashed curves). The FTC pairs are aligned according to their CF, the approximate value of which is indicated for each group . (The horizontal position of FTCs is arbitrary).

CF of the IHC FTCs would be lowered compared with the CF of the normal FTC, whereas for low frequency fibres there would be an upward shift in CF after OHC loss. These CF changes are comparable with those observed in acute and reversible FTC changes (e.g. EVANS 1974c).

7.5 INNER AND OUTER HAIR CELL INTERACTION.

The results of the present study, along with other evidence (reviewed in section 1.3a), support the idea that the presence of the OHCs is required for the low thresholds and sharp tuning properties of normal cochlear fibres. Because most afferent fibres originate at the IHCs, an interaction between inner and outer haircells is required. Models and suggestions for the low threshold cochlear filtering mechanism have been proposed which explicitly invoke an interaction; other models could possibly involve an interaction. This section briefly examines how compatible such models are with the results of the present study.

A number of interpretations of the present results are possible.[8]

a). That normally, the IHCs are sharply tuned and of low threshold but that the physical loss of OHCs has interfered with the mechanical input to the IHCs.

b). That the IHCs are normally sharply tuned as a result of some active interaction (hydromechanical or electrical) with the OHCs, and that the loss of the OHCs eliminates their important influence.

c). That the OHCs themselves are sharply tuned and of low threshold, and that cochlear fibre responses reflect OHC activity by means of an interaction between the innervation of the OHCs and the majority of the cochlear afferent fibres.

a), b), and c) are discussed more detail below.

a). Although it does not seem likely that OHC loss would alter the gross mechanical properties of the BM vibration (e.g. as measured from the scala tympani side of the BM), even small changes in the physical structure of the organ of Corti must be considered as a possible cause of the deterioration in threshold and tuning properties if mechanical or hydromechanical processes are involved in cochlear filtering (i.e. in addition to the coarse mechanical tuning of the BM). Thus in the models of cochlear filtering proposed by STEELE (1973), HELLE (1974), ZWICKER (1974), and CRANDELL (1975), in which the IHCs are stimulated by sub-tectorial fluid streaming, although

---

8 This is excluding the possibility that the IHCs are exclusively responsible for the normal tuning and threshold properties of cochlear fibres, but that when they remain in animals after kanamycin treatment, they are functionally abnormal as a result of the direct action of kanamycin upon them.

the damage to the OHCs may not affect the gross mechanics which the models invoke, it is clearly possible that the loss of stereociliar attachment to the tectorial membrane could have an affect on the maintenance of the gap between the reticular lamina and the tectorial membrane. This could influence the important subtectorial fluid flow invoked in these models.

In any mechanism of cochlear filtering which involves a micro-mechanical input to the IHCs, the physical loss of OHCs could be expected to deleteriously influence that mechanism.

b). A number of models of cochlear filtering have been proposed in which the OHC responses can interact with the activity of the IHCs. Three [9] types of interaction are most commonly invoked: hydromechanical, electrical or neural interaction.

The hydromechanical models of STEELE, of HELLE, of ZWICKER, and of CRANDELL, mentioned above, do not explicitly involve OHCs (except in CRANDELL'S model where the arrangement of the stereocilia of the OHCs serves to prevent longitudinal fluid streaming). However, assuming that the mechanical properties of the stereocilia are important in maintaining the gap between the reticular lamina and the tectorial membrane, the recent suggestion by FLOCK (1977) that the OHC stereocilia may be capable of changing their stiffness, could allow the OHCs to actively influence the mechanical relationship between the organ of Corti and the tectorial membrane, and therefore the proposed hydro-mechanics of the models. Further speculation would not be useful, but it may be worth noting that if the physiological activity of the OHCs can control the properties of their stereocilia, these hydro-mechanical models of filtering may become more compatible with one of the characteristics of the sharp cochlear filter, namely its physiological vulnerability.

Models involving direct electrical interaction between outer and inner hair cells have been proposed by DALLOS & HARRIS (1977) and by MANLEY (1977). They both involve the attractive proposition that hair cells could be combined mechano-electro-receptors, not an unreasonable hypothesis considering how closely related true electro-receptors and hair cells are (BENNETT 1970).

The model of DALLOS & HARRIS depends on the inner hair cells already being very sharply tuned (as sharply tuned as normal fibre responses), and being sensitive to the receptor potentials of the OHCs. The function of the OHCs is to extend the intensity range over which frequency selectivity is effective. Figure 7.7 shows the contribution of the inner and outer hair cells to the FTC. Note that because the IHCs are sharply tuned, the influence of the OHC receptor potentials need not be very frequency specific.

Needless to say, the physiological results of DALLOS et al (1977) in

---

[9] The optical system of NIEDER (1974) is not under consideration here.

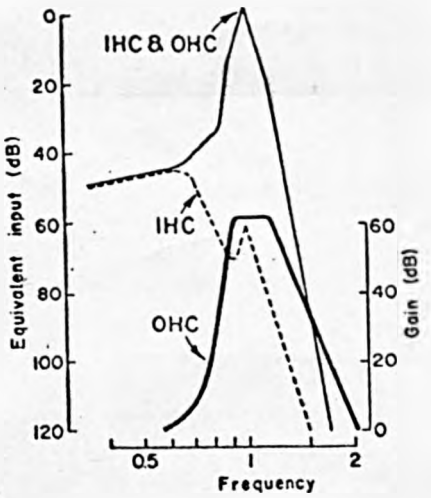


Figure 7.7 Diagram from DALLOS & HARRIS (1977) showing, according to their model, the contributions of inner and outer hair cells to the normal tuning characteristics of cochlear fibres. The thick, solid curve indicating the influence of the OHCs, is produced by subtraction of a 'typical' IHC response (dashed curve) from the normal FTC (IHC & OHC).

kanamycin treated chinchillas support the model, but are at variance with the results from this present study in which "IHC FTCs" are not sharply tuned. In this case, the influence of the OHCs on the IHCs would have to be very frequency selective. The question as to whether this could be accomplished by an electrical field via wide endolymphatic spaces cannot be answered although clearly the 'source' of an electrical field at the OHC level could be very localized judging from the narrow receptor potential tuning of (presumed)[10] OHCs by (RUSSELL & SELLICK 1977).

In MANLEY'S (1977) model, a DC potential change (-SP) caused by the OHCs (which dominate SP production, DALLOS et al. 1972) inhibits the IHC's response to mechanical stimulation. The model assumes that the inner hair cells are mechanically stimulated at all intensities. The effect of removing the inhibitory influence of the OHCs should be to produce broadly tuned and low threshold FTCs. This sort of tuning curve has never been found in the present study from regions of outer hair cell loss, and indeed has rarely been found at all.

A neural interaction between inner and outer hair cells has been proposed by ZWISLOCKI (1974, 1975, 1977; ZWISLOCKI & SOKOLICH, 1974). The model relies heavily on an inhibition (explicitly synaptic) of the output from the IHCs by the OHCs. One[11] reason for this model being unattractive is that, unfortunately, no suitable synapses appear to exist.

c). The possibility that the OHCs are exclusively sharply tuned and of low threshold, and that a direct information transfer between outer spiral fibres and the majority of cochlear afferents can occur is also unattractive. Such interactions cannot be seriously entertained until there is a positive indication that neural interaction is possible (e.g. a demonstration of synaptic or ephaptic structures). In any case, there is evidence to suggest that in the normal cochlea the IHCs may be sharply tuned (RUSSELL & SELLICK 1977a, b) and thus any proposed influence of the OHCs on the cochlear filtering mechanism is most likely to be at the IHC level and not more central (e.g. habenula perforata). It may, however, be premature to use RUSSELL & SELLICK'S data as conclusive evidence that IHCs are as sharply tuned as cochlear fibres. The relationship between the receptor potential tuning found by these authors and cochlear fibre tuning may not be a direct one, and the possiblity that the observed receptor potential tuning was artificially enhanced by the presence of the electrode in the hair cell has yet to be tested (RUSSELL personal communication). Nevertheless, the notion that the IHCs are directly influenced by the OHCs is attractive.

---

10 RUSSELL & SELLICK (1977a) have recorded from hair cells which they suspected, but could not verify, to be OHCs. The tuning of the receptor potentials of such cells was very narrow ($Q_{10\ dB}$ up to 25).

11 See also section 1.3e for further arguments against this model.

Although the results of the present study have indicated the important contribution of the OHCs to the low thresholds and sharp tuning of afferent cochlear fibres, the exact role of the OHCs still remains undefined.

7.6 AN ANIMAL MODEL OF SENSORINEURAL HEARING LOSS OF COCHLEAR ORIGIN.

Because chronic hair cell damage is a characteristic feature of both kanamycin treated GPs and many forms of sensorineural hearing loss of cochlear origin (BREDBURG, 1968; SCHUKNECHT, 1974), these animals may be useful models of this form of hearing loss.

The present physiological findings of a deterioration in the threshold and tuning properties of cochlear fibres which accompany OHC loss, appear to have parallels in psychophysical studies in patients with sensorineural hearing loss of cochlear origin. Before drawing any comparisons it is necessary to examine a) the closeness of the analogy between GP cochleas with hair cell loss and pathological human cochleas (this is considered in section 7.6a), and b) the validity of comparing physiological data with the results of psychophysical studies (across species). With regard to the latter, section 7.6b reviews the evidence which suggests that the normal, psychophysically measured, frequency selectivity of the auditory system is determined at the cochlea and is therefore closely related to the tuning of cochlear fibres.

In section 7.6c, the changes in cochlear fibre tuning found in the present study are compared with the changes in psychophysically determined, frequency selectivity which are known to accompany hearing losses of cochlear origin. Section 7.6d briefly mentions other characteristic features of sensorineural hearing loss of cochlear origin which may have neural correlates at the cochlear level.

7.6a THE ANALOGY BETWEEN GP COCHLEAS WITH HAIR CELL LOSS AND PATHOLOGICAL HUMAN COCHLEAS.

The most obvious comparisons can be made between GP cochleas damaged by aminoglycoside poisoning and human cochleas which have also been damaged with the same agents. Post mortem examinations of such cochleas have demonstrated hair cell loss to be the essential lesion, particularly in the basal turn (JØRGENSEN & SCHMIDT, 1962; LOWRY et al. 1973) and more specifically the loss of OHCs (BENITEZ et al. 1962; MATZ et al. 1965). Cochlear hearing loss caused by aminoglycoside poisoning does not seem to be a particular case; symptoms were often exhibited by these patients which are typical of cochlear hearing losses in general, e.g. recruitment (an abnormally rapid increase in

loudness with intensity; SATLOFF et al. 1964; LIDÉN, 1953). Tinnitus of the type associated with other cochlear hearing losses has also been demonstrated in patients after aminoglycoside poisoning (JØRGENSEN & SCHMIDT, 1962; FROST et al. 1960; LIDÉN, 1953; MATZ et al. 1965).

Furthermore, other types of cochlear hearing loss have been shown to involve hair cell loss. As well as aminoglycosides, other ototoxic agents can cause OHC loss in humans e.g. ethacrynic acid (MATZ, 1969) and nitrogen mustard (SCHUKNECHT, 1974). Other forms of human endorgan deafness often involve loss of OHCs e.g. acoustic trauma, sensory presbyacusis and certain forms of hereditary deafness (e.g. Alports syndrome) (GUILD, 1931; IGARASHI et al. 1964; BREDBERG, 1968; SCHUKNECHT, 1974; McGILL & SCHUKNECHT, 1976).

The observations mentioned above suggest that GPs with OHC loss can be regarded as a useful animal model of many types of deafness of cochlear origin. Caution must be exercised to avoid making the sweeping generalization that all types of sensorineural hearing loss of cochlear origin involve OHC loss. This, for example, is not the case in temporary hearing losses such as in attacks of Ménières disease or during the acute administration of ototoxic drugs (e.g. salicylates and certain antibiotics and diuretics) where there is no hair cell loss. However, the post mortem histological investigations which were carried out in patients with chronic cochlear pathology, clearly indicate that hair cell loss, particularly OHCs (BREDBERG, personal communication), is a very common characteristic.

7.6b THE RELATIONSHIP BETWEEN NORMAL PSYCHOPHYSICAL AND NEURAL FREQUENCY SELECTIVITY.

One measure of the frequency selectivity of the auditory system is the critical bandwidth. This can be expressed as the frequency bandwidth within which signals sum and interact in various ways (ZWICKER, 1952, 1954; GÄSSLER, 1954; TERHARDT, 1968, 1970; GREENWOOD, 1961; McCELLAND & BRANDT, 1969; review: SCHARF, 1970). Early critical band measures were taken to represent the bandwidth of a rectangular filter centred on the frequency of interest. Investigation of the shape of the auditory filters showed them to be (on a linear frequency scale) approximately Gaussian in shape (HOUTGAST, 1974; PATTERSON, 1974, 1976), and in this respect they are of similar shape to the tuning curves of cochlear fibres (EVANS & WILSON, 1973; EVANS, 1977). EVANS & WILSON (1971, 1973; EVANS, 1977) have made quantitative comparisons between the critical bandwidths of the auditory filter and analogous bandwidths of cochlear fibres, that is, their effective bandwidths (i.e. the bandwidth of an equivalent rectangular filter). Figure 7.8 , taken from EVANS & WILSON 1973, shows the comparison between the effective bandwidths of cat cochlear

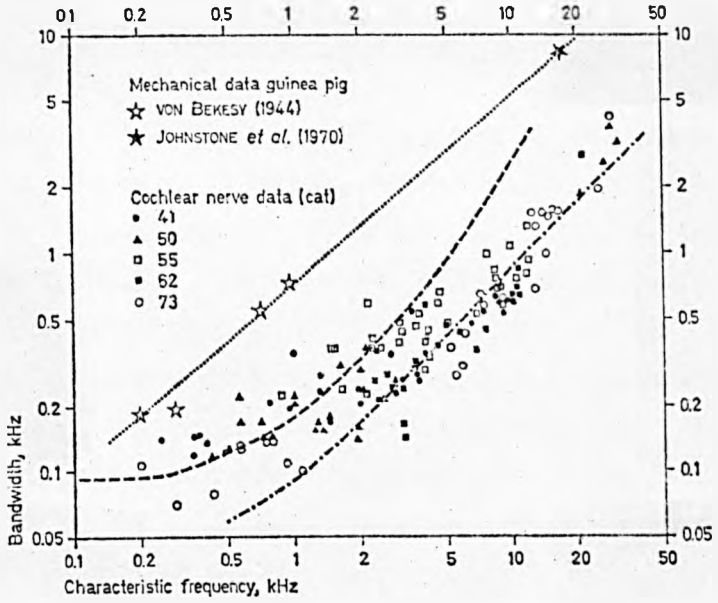


FIGURE 7.8 (from EVANS 1975c, after EVANS & WILSON 1973). Effective bandwidths computed for 140 cat cochlear fibres from 5 cats, plotted against their characteristic frequencies. Dotted line = effective bandwidths computed for basilar membrane response curves of VON BÉKÉSY (1944) and JOHNSTONE et al. (1970); dashed line = critical band measures for man (ZWICKER et al. 1957); dotted and dashed line measurements of human psychophysical frequency resolving power expressed as effective bandwidth for auditory gratings.

fibres and psychophysical measures of the frequency selectivity of the auditory system. There is tolerable agreement between the psychophysical measures of critical bandwidth (dashed line) and the effective bandwidths of cat cochlear fibres and this led EVANS & WILSON to hypothesize that the frequency selectivity of the auditory system was already determined at the cochlear level (EVANS & WILSON, 1973).

Support for this hypothesis has come from measures of the frequency resolving power of cat cochlear fibres and of human subjects with comb-filtered noise masking paradigms (see figure 7.9 for psychophysical technique). There was good agreement between the ability of the auditory system to separate out peaks and valleys of such a masker, and the ability of cochlear fibre responses to resolve the spacing of such a grating (WILSON & EVANS, 1971; EVANS & WILSON, 1973).

Other measures of the frequency selectivity of the auditory system are psychophysical tuning curves. The curves derived using forward and simultaneous tone on tone masking paradigms[12] show a similarity to cochlear fibre tuning curves (e.g. ZWICKER, 1974; VOGTEN, 1974; HOUTGAST, 1974; HOEKSTRA & RITSMA, 1977; LESHOWITZ & LINDSTROM, 1977; WIGHTMAN et al. 1977). Although some of the psychophysical tuning curves derived using simultaneous masking methods show irregularities in the curve at the test tone frequency (W shaped tips to the curves), the gross resemblance of the curves to FTCs of cochlear fibres adds support to the idea that the frequency selectivity of the auditory system is determined at the cochlear nerve level.

The notion that psychophysical and cochlear nerve effective bandwidths are equivalent has not been fully substantiated by measurements of behavioural critical bandwidths in the cat (PICKLES, 1975). A comparison of the psychophysical and physiological measurement (in the same animal PICKLES & COMIS, 1976) indicated that behavioural critical bandwidths were twice as large as in the equivalent cochlear fibre tuning. If accepted at face value, these results suggest that a direct correlation between the frequency selectivity of the auditory system and cochlear fibre tuning may not be valid, and that perhaps the critical band occurs as the result of convergence of two or more cochlear fibre bandwidths. This implies that higher neural levels are involved (PICKLES & COMIS, 1976). However, the effective bandwidths of cells in the cochlear nucleus are not wider than those of the cochlear nerve in the same cat (EVANS & PALMER, to be published). It has also been pointed out (EVANS, 1977) that the possible effects of efferent activity cannot be

---

12 Psychophysical tuning curves derived from a temporal masking paradigm e.g. in the pulsation threshold technique (HOUTGAST, 1974, DUIFHUIS, 1977) appear to reflect filtering processes at a higher neural level than the cochlear nerve. Such masking curves exhibit areas of sideband suppression which could be related to lateral inhibition found, for example, in the dorsal cochlear nucleus.

ignored. The cochlear fibre bandwidths measured in the anaesthetized animal may be narrower than normal (stimulation of the efferent pathway decreases the frequency selectivity of cochlear fibres, WIEDERHOLD, 1970).

It is also important to ask whether the behavioural test used in PICKLES'S study was exactly equivalent to the human psychophysical paradigm. This is a particularly relevant question in the light of another comparison between psychophysical and cochlear fibre tuning in the same species (chinchilla) by DALLOS et al. (1977). In that study, behavioural psychophysical tuning curves, using forward and simultaneous masking techniques (McGEE et al. 1976) were found to be much more sharply tuned than the corresponding cochlear fibre tuning. For example at 5 kHz, the $Q_{10\ dB}$ of cochlear fibres was, on average, 4, whilst the psychophysical tuning curves had $Q_{10\ dB}$ values of 8.

The two above cases of conflicting evidence could perhaps be resolved by using alternative behavioural methods for determining the bandwidth of the auditory filter, in the same animal.

There are thus some considerations that should be made before rejecting the hypothesis that the frequency selectivity of the auditory system is determined at the level of the cochlear nerve. In whatever way the critical band ultimately relates to the cochlear fibre tuning, it is clear that the drastic deterioration in tuning that can occur at the cochlear fibre level (e.g. in acute cochlear hypoxia, EVANS, 1972) will almost certainly cause a deterioration of the frequency selectivity of the whole system.

7.6c FREQUENCY SELECTIVITY IN COCHLEAR PATHOLOGY.

A number of physiological studies have demonstrated a deterioration in the tuning[13] of cochlear fibres as a result of cochlear pathology (KIANG et al. 1970; EVANS 1972, 1974a,b, 1975b,c, 1976a; ROBERTSON & MANLEY, 1974). It was largely the result of such studies that led to the hypothesis that one of the invariant features of human cochlear pathology should be a deterioration in frequency selectivity. PICK et al. (1977) have tested this hypothesis by measuring the frequency resolving power of normal subjects and patients with sensorineural hearing loss of cochlear origin using a comb filtered noise masking technique (HOUTGAST, 1974; PICK et al. 1977). Figure 7.9 illustrates the experimental paradigm; the method is fully described in the figure legend. The results of this study clearly indicated that a deterioration in the frequency selectivity of the auditory system accompanies

---

[13] As would be expected from the changes in bandwidth of the FTC tip, the effective bandwidths of cochlear fibres also increase under conditions of cochlear pathology (EVANS, 1976b).

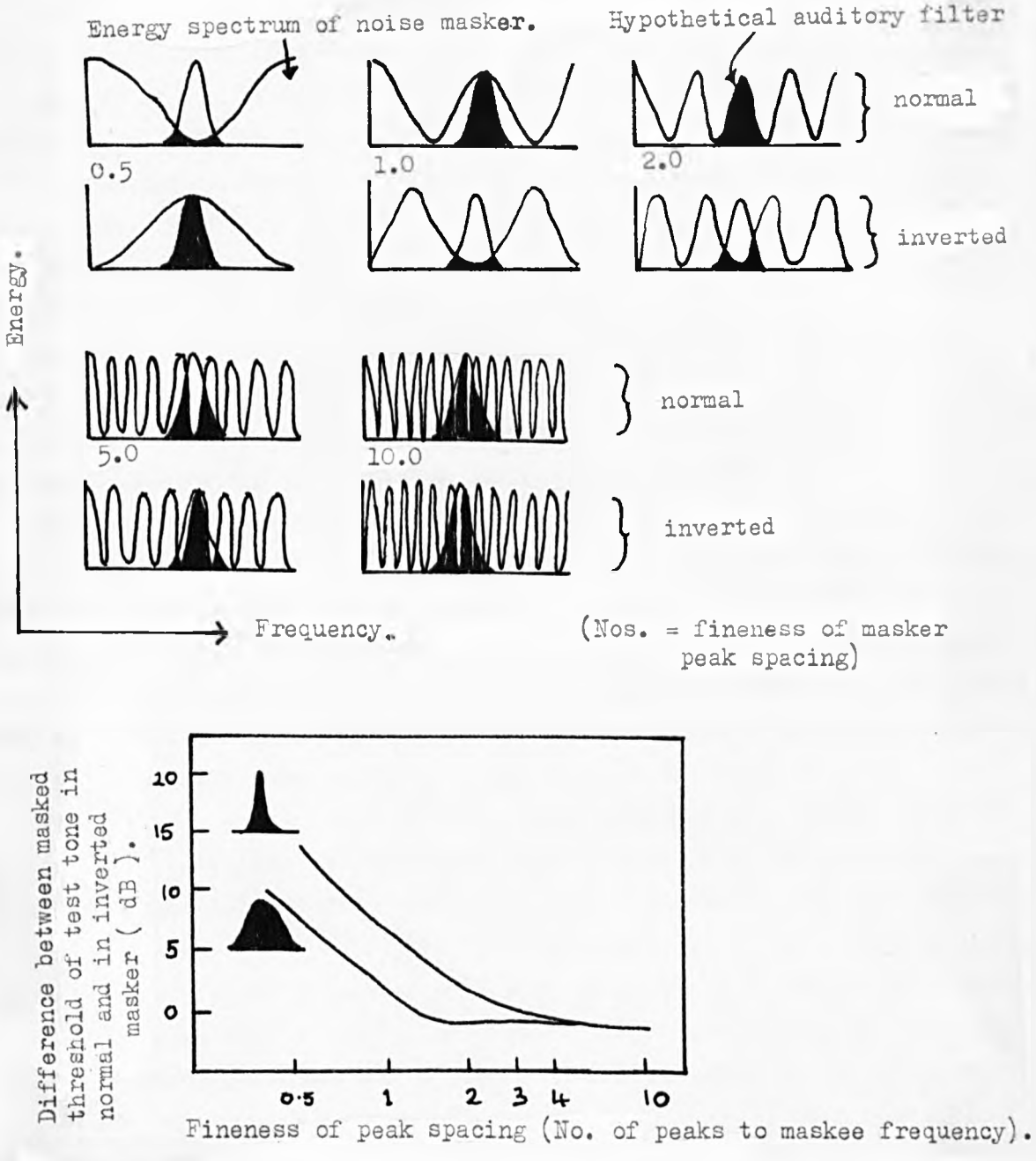


Figure 7.9 The psychophysical measurement of frequency resolving power, using comb-filtered noise masking. Comb filtered noise is produced by mixing noise with a delayed version of itself, giving a cosinusoidal distribution of energy along a linear frequency scale. Tone thresholds are measured in the presence of the noise, both where a 'dip' in the energy spectrum coincides with the test tone frequency and, in the inverted case, where a peak in the energy spectrum coincides with the test tone frequency. The difference between the masked tone thresholds in these two situations depends on how much noise (masking) energy falls within the bandwidth of the auditory filter. The maximum difference will occur when the peak spacing of the noise is extremely wide compared to width of the auditory filter. At very close peak spacing, the difference between masked thresholds will be close to zero because inverting the noise will produce practically no difference in energy falling within the auditory filter. The relationship between the difference in masked threshold, and the fineness of peak spacing of the masker is shown in the lower part of the figure. It illustrates how the function reflects hypothetical auditory filters of different bandwidths. (Figure after PICK & WILSON.)

hearing losses of cochlear origin.

Other evidence of a deterioration in the frequency selectivity of the auditory system in sensorineural deafness of cochlear origin comes from measurements of psychophysical tuning curves (e.g. tone on tone masking curves) in patients with cochlear pathology. The curves from such patients show an elevated threshold, accompanied, if the threshold elevation is considerable, by a large increase in their bandwidth (LESHOWITZ, 1976; LESHOWITZ & LINDSTROM, 1977; CARNEY & NELSON, 1976; SCHORN et al, 1977; WIGHTMAN et al. 1977; HOEKSTRA & RITSMA, 1977).

Many early psychophysical studies in patients with assumed cochlear pathology can now also be interpreted in terms of a deterioration in the frequency selectivity of the auditory system. These studies are only of indirect relevance and are reviewed in Appendix A (pg 168).

The results of the present study, showing that cochlear fibres with elevated thresholds resulting from hair cell damage had increased FTC bandwidths (X 5 on average), serve to corroborate the previous physiological findings. Furthermore, the present results in chronically damaged GP cochleas are more appropriate for comparison with most types of human (chronic) cochlear pathology (most of the previous findings, except those of KIANG et al. 1970, were based on acute cochlear pathology).

In addition, the present data have indicated the relationship between the threshold elevation and cochlear tuning. For fibres with CFs above about 2 kHz, the relationship between threshold elevation and the deterioration in the FTC (10 dB) bandwidth was non-linear, that is to say, the bandwidth changed relatively little until some 40-50 dB of threshold elevation occurred (figures 4.27 & 4.30). This non-linear relationship has parallels in psychophysical measurements of the frequency selectivity in patients with hearing loss of cochlear origin. Thus PICK et al. (1977) have obtained data from psychophysical measurements of the frequency resolving power in which, particularly for higher frequencies, the effective bandwidths of the derived filter functions increased little until the threshold elevation was about 40-60 dB. This in turn may be related to the finding of HOOD & POOLE (1971) and PRIEDE & COLES(1976) that deterioration in speech intelligibility in patients with cochlear hearing loss is not a linear function of threshold elevation but becomes marked only for elevations of threshold above 30-40 dB.

Finally, there are also striking similarities between the tuning of single cochlear fibres in the present study and measurements of psychophysical tuning curves in patients with similar threshold elevations (LESHOWITZ, 1976; LESHOWITZ & LINDSTROM, 1977; WIGHTMAN et al. 1977; SCHORN et al. 1977; HOEKSTRA & RITSMA, 1977). These data have in common with the physiological data of the present study, progressive loss of the low threshold, sharply tuned tip of the tuning curves until only the high threshold, broadly tuned

segment remains. An example of this is shown in figure 7.10.

7.6d OTHER CHARACTERISTICS OF HEARING LOSS OF COCHLEAR ORIGIN WHICH MAY HAVE NEURAL CORRELATES AT THE COCHLEAR LEVEL.

Other characteristic features of sensorineural hearing loss of cochlear origin may have neural correlates at the cochlear nerve level, and the kanamycin treated GP could prove a useful model for the systematic investigation of some of these features.

Tinnitus[14] is frequently associated with hearing loss of cochlear origin including that caused by aminoglycoside poisoning (JØRGENSEN & SCHMIDT, 1962, FROST et al. 1960; LIDÉN 1953; MATZ et al. 1965). One possibility is that tinnitus may be related to spontaneous over-activity in particular groups of cochlear fibres; it is possible that some correlate of it could be detected in cochlear fibre recordings. The present study, as did that of KIANG et al. (1970) in kanamycin treated cats, found no evidence of overactivity, but rather the converse, that many pathological fibres had zero spontaneous rates of activity. KIANG et al. tentatively suggested "it may be that it is this absence of activity that results in a subjective sensation of sound", or "not the loss of activity as such but the pattern of the loss". Alternatively perhaps individual fibres or groups of fibres with normal rates of spontaneous discharge but set against a background of fibres with depressed discharge could give rise to a percept of tinnitus. However, a search for neural correlates of tinnitus would be best carried out in animals in which behavioural tests (if feasible) indicate that tinnitus was present.

Recruitment (an abnormally rapid increase in loudness with increase in intensity) is also frequently found in patients with sensorineural hearing loss of cochlear origin including those with aminoglycoside poisoning (LIDÉN, 1953, SATLOFF et al. 1964). A useful model of loudness recruitment has been proposed (EVANS 1972, 1975b, c) based on the overlap of the abnormally broadly tuned cochlear fibre FTCs found in cochlear pathology. Because each of these fibres responds to a wide range of stimulus frequencies, a suprathreshold stimulus will excite a very large population of fibres (see figure 8.10) and this may give rise to an abnormally rapid growth in loudness.

Also related to an increase in loudness could be the slopes of the fibre discharge rate versus intensity functions. EVANS (1975b) suggested that a rapid increase in loudness could result from abnormally steep functions. He found that after acute cochlear insult that some fibres had abruptly rising rate functions. However KIANG et al. (1970) reported that the fibres from

---

[14] The many forms of tinnitus which are not cochlear in origin (e.g. outer & middle ear or centrally generated) are not under discussion.

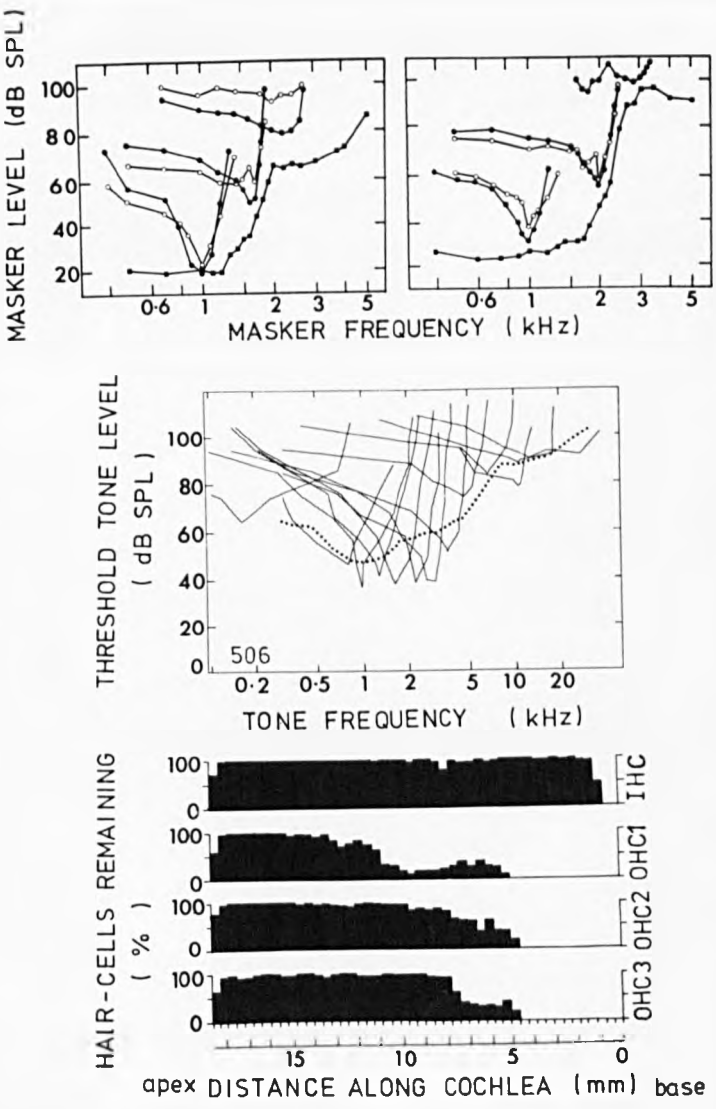


Figure 7.10 Comparison between psychophysical "tuning curves" in patients with cochlear hearing loss, and physiological FTCs from GPs with specific cochlear pathology. The upper panels show, from a study by Wightman et al. (1977), psychophysical FTCs for two hearing impaired subjects, determined by simultaneous (open circles) and forward masking (filled circles) paradigms. Audiometric thresholds are indicated by the filled squares. Lower part, from the present study, FTCs of GP 506 with neural threshold loss similar to the patients audiometric losses.

kanamycin damaged cochleas did not have abruptly rising functions. In the present study such functions have not been systematically measured. However some pathological fibres were found in which the mean firing rate of the fibre increased from threshold to saturation with less than a 10 dB increase in stimulus intensity. Further investigation is required, but whatever possible neural substrate for recruitment is found will need cautious interpretation; it is still unclear (at least under certain conditions) how intensity changes are encoded at the cochlear nerve level (PALMER 1978).

It has been suggested (e.g. EVANS 1972, 1975b,c, 1977) that the deterioration in frequency selectivity (as a result of the increased FTC bandwidths of cochlear fibres with threshold elevation) is a contributory cause of the poor speech intelligibility of patients with sensorineural hearing loss of cochlear origin. With particular regard to speech signals it is of interest to ask whether the temporal coding ability of the pathological cochlear is also impaired. Preliminary studies on the phase locking ability of high threshold fibres in kanamycin treated GPs indicate that there may be no significant deterioration in this aspect of temporal coding. However, further systematic investigation is required, and again, animal models of cochlear hearing loss such as kanamycin poisoning GPs may be a potentially useful substrate.

CHAPTER 8.

DISCUSSION: COCHLEAR ACTION POTENTIAL (CAP) STUDIES.

8.1 THE COCHLEAR ACTION POTENTIAL (CAP) AUDIOGRAM.

8.2 THE CAP AMPLITUDE : INTENSITY FUNCTION.

8.3 THE CAP AMPLITUDE : INTENSITY FUNCTION IN COCHLEAR PATHOLOGY.

8.4 THE CAP LATENCY : INTENSITY FUNCTION.

8.1 THE COCHLEAR ACTION POTENTIAL (CAP) AUDIOGRAM.

It was noted in the results section (5.1) that there was some scatter in the data relating the CAP thresholds to the minimum thresholds of cochlear fibre responses (figure 5.10). There are a number of possible causes for the scatter. Firstly, for the single fibre responses, the range of minimum thresholds at any CF can be up to 30 dB in an individual animal (KIANG, 1968, cat; EVANS, 1972, GP). Secondly, the threshold criterion for a cochlear fibre response (detection, using auditory cues, of an increase in firing rate above the spontaneous level) is likely to change depending on the fibre's spontaneous rate of activity. Thirdly, the CAP threshold determination will be influenced by the signal to noise ratio of the recorded response, which may differ from animal to animal depending on small differences in gross electrode placement.

Apart from the scatter, it was noted in the results section that two trends are apparent in the data of figure 5.10. At low frequencies (below 2-3 kHz) the CAP threshold is often higher than the single fibre thresholds. This is probably the result of the poor synchrony of discharge of fibres in response to stimuli of low frequency, which makes the CAP less obvious than that evoked by stimuli of high frequency. The detection threshold for low frequency CAPs will be somewhat raised as a consequence.

The other trend in the data is evident at high frequencies where many cochlear fibres appear to have higher minimum thresholds than the CAP thresholds. One possible explanation for this has been investigated and excluded. That is, that when investigating the CAP thresholds for the high frequency cut-off slope of the audiogram, perhaps energy spread to lower frequencies than the stimulating frequency may stimulate the most sensitive portion of the audiogram (i.e. at 8-10 kHz) and thus give a false, lower threshold value. However, appropriate high pass filtering of the stimulus to avoid the possibility of stimulating the more sensitive lower frequencies revealed no change in the CAP threshold values. This is illustrated in figures 8.1 & 8.2. In figure 8.1 a series of 5 CAP audiograms from one GP are shown, plotted before, during, and after high pass filtering. Various filter cut-off frequencies were used (7, 12 & 15 kHz; the cut-off slopes were greater than 100 dB/octave) and it can be seen that the CAP threshold values for the high frequencies are unchanged. In figure 8.2, the CAP audiograms are plotted with (dotted curve) and without filtering the stimuli with a 1/3 octave band pass filter centred on the stimulus frequency. Again, there is no alteration in the CAP threshold values obtained for the high frequencies.

Another possible explanation for CAPs having lower thresholds than

fig. 8·1

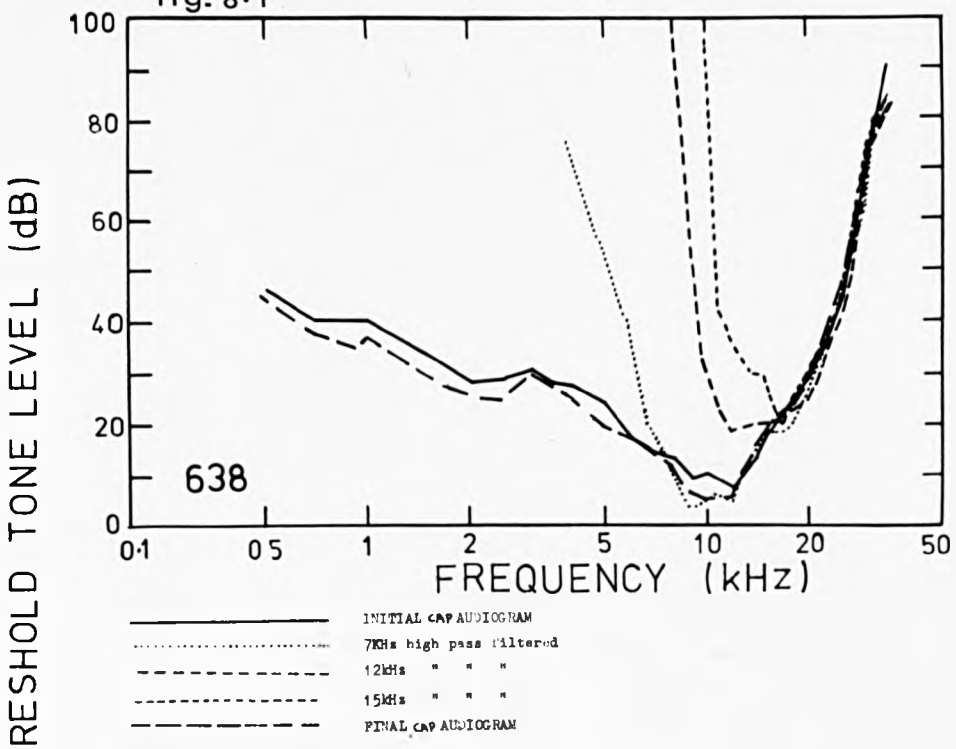


fig. 8·2

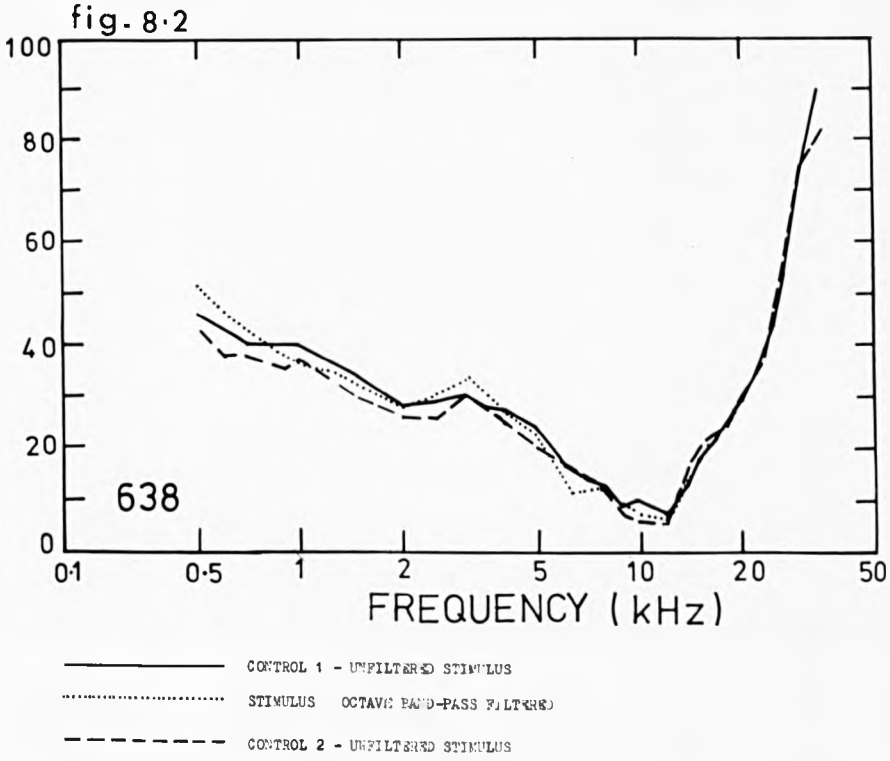


Figure 8.1 CAP audiograms measured from a normal GP with and without high-pass filtering of the stimulus (4 ms tone pip, 2 ms rise/fall). See key for filter cut-off values.

Figure 8.2 CAP audiogram measured from a normal GP with the stimulus (4 ms tone pip) unfiltered (continuous & dashed curves) and filtered (dotted curve) with a 1/3 octave band-pass filter.

cochlear fibre thresholds is that some of the single fibre thresholds could have deteriorated during the course of an experiment; in most cases the CAP thresholds were not determined at the same time as the cochlear fibre responses, but 1-6 hours later. A further possible reason is that because the CAP is the result of synchronous activity of many cochlear fibres, it is in effect an averaged response. As such, it could yield a lower threshold value than that of any individual cochlear fibre, which is ascertained without averaging. This is particularly possible for high frequency stimuli, which produce the most synchronous cochlear fibre discharges, and thus where the CAP best represents cochlear fibre activity.

Given the limitations already discussed, the demonstrated relationship between the CAP audiogram and the minimum thresholds of cochlear fibre responses is good evidence that CAPs evoked by a tone pip are the result of activity of cochlear fibres with CFs near to the frequency of the stimulus. Further evidence to indicate that responses near threshold are associated with events at specific points along the cochlea is the increasing latency of the N_1 of the CAP with a decrease in stimulating frequency. This can be observed in figure 8.3 which shows, from a normal GP, a series of CAP responses evoked by tone pips of various frequencies. Each trace is an average of 100-150 responses; because successive stimuli are in random phase, any cochlear microphonic component is largely eliminated. The intensities of the stimuli were within 15 dB of the visually determined CAP threshold.

It was not possible to measure the absolute latency values of these responses because of the uncertainty of when, on the stimulus rise time, the response is evoked. However, the relative latency values are consistent with latency measurements of single cochlear fibres to click stimulation (EVANS, 1972). For example, in GP cochlear fibres, the difference in response time between fibres with CFs of 20 kHz and 5 kHz is approximately 0.6 ms. The latency difference between the N_1 response to a 20 kHz stimulus and to a 5 kHz stimulus is also approx. 0.6 ms. However, although the N_1 latencies in figure 8.3 are compatible with cochlear fibre data for high frequencies (above approximately 2 kHz) the N_1 latencies for low frequency signals are not long enough. For example, the N_1 of a 500 Hz evoked CAP occurs approximately 0.5 ms earlier than the latency of cochlear fibres with CFs of 500 Hz suggests it should. In figure 8.3 it can be seen that the N_1 wave in the CAP evoked by low frequency stimuli, precedes the largest, second response peak (N_2). This small N_1 is assumed to be the result of some spread of high frequency energy in the stimulus, to basal regions of the cochlea.

To test this idea that the N_1 to low frequency stimulation is an artefact of high frequency energy spread, the tone stimuli were made more

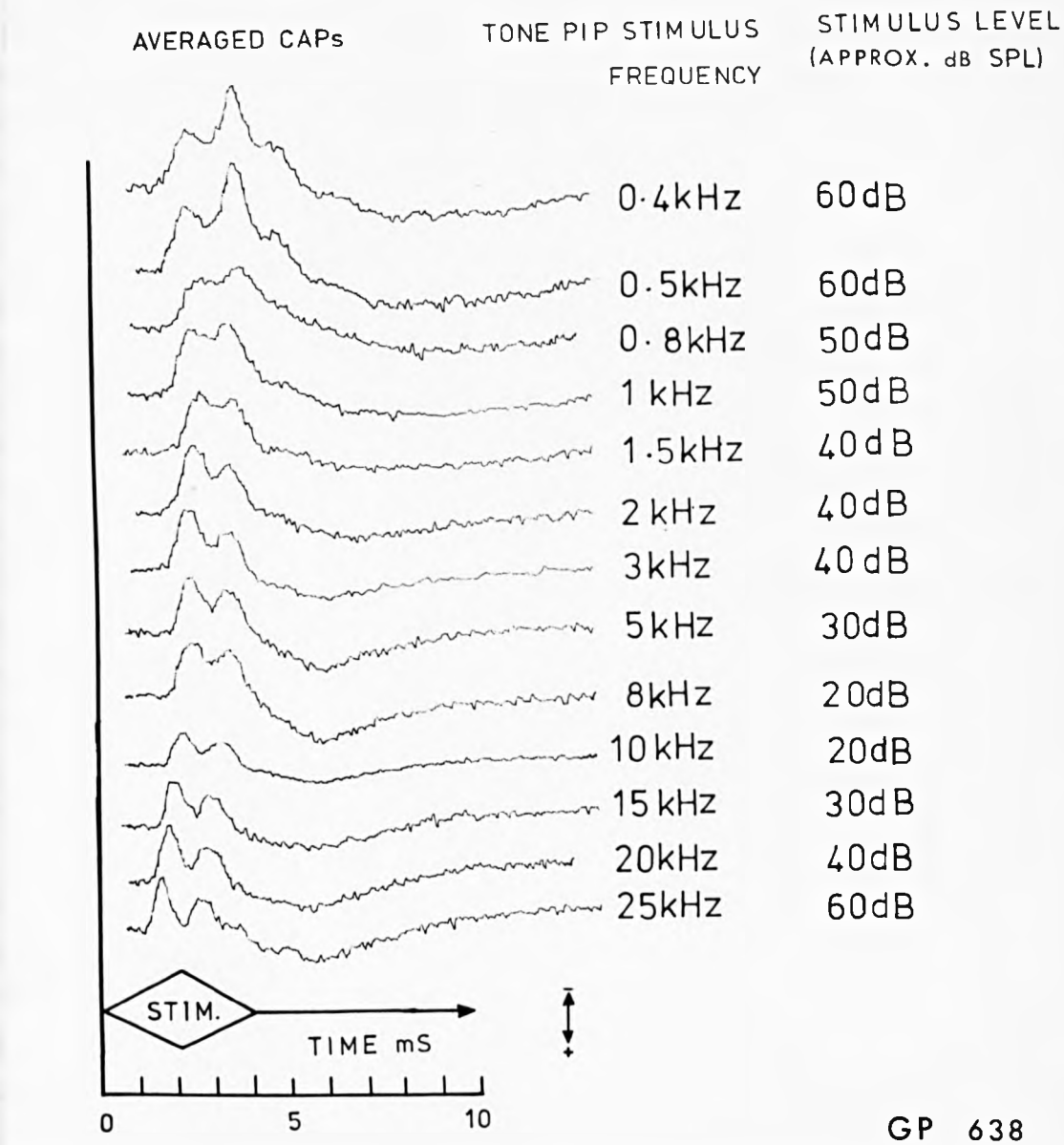
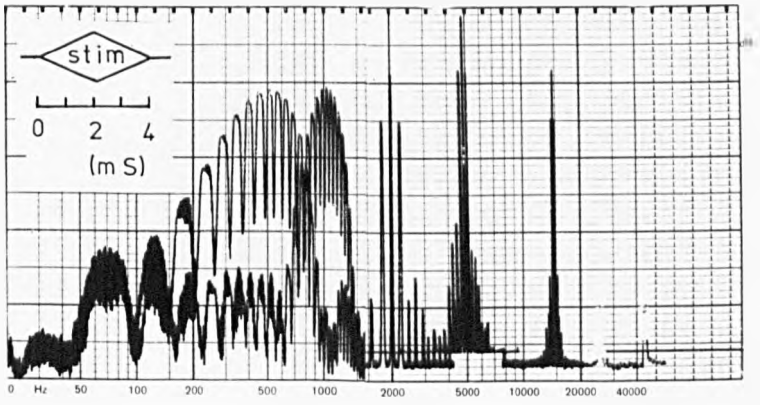


Figure 8.3 Near threshold, tone evoked CAPs (averaged) recorded from near the round window in a control GP. The frequency and intensity of the stimuli are indicated (right). The stimulus was 4 ms tone pip with 2 ms rise/fall times.

frequency specific by filtering them through 1/3 octave band-pass filters (centred on the stimulus frequency). The stimuli then had less energy spread to other frequencies whilst retaining their necessary impulsive properties, essential for evoking a CAP response. Figure 8.4 shows the energy spectra of a sample of stimuli with and without such filtering. For frequencies above 2 kHz the filtering does little to change the stimulus spectrum. This is borne out in figure 8.5 which shows the averaged CAP responses evoked by the 1/3 octave band-pass filtered stimuli. Above 2 kHz the responses are essentally similar to those produced by unfiltered stimuli (figure 8.3). For frequencies of stimulation below 2 kHz, the CAP responses evoked by the filtered tone pips are of longer latency than for the unfiltered stimuli. In figure 8.6, plotted against frequency, are the latencies of the N_1 , N_2 and N_3 peaks of the CAPs shown in figure 8.3, and also (in the dotted line) the latencies of the CAPs evoked by the filtered stimuli (from figure 8.5). The latencies of the responses to the filtered stimuli have been corrected for the stimulus delay through the band-pass filter. The dashed parts of the N_1 and N_2 latency curves indicate the peaks of maximum amplitude. It is these peaks which correspond, in latency, to the more frequency specific responses obtained using 1/3 octave band-pass filtered stimuli (dotted line). Thus the dominant response in the CAP evoked by stimuli below 1 kHz is of the appropriate latency to suggest that it represents the activity of cochlear fibres with CFs below 1 kHz.

The limitations of using tone pips (2 ms rise/fall time) as stimuli for assessing the response threshold of low frequency, apical areas of the cochlea have been stated. Nevertheless, such a tone pip seems to be nearly optimal for eliciting a frequency specific CAP response. As mentioned previously, the amplitude of the peaks in a CAP will very much depend on the synchrony of firing in a population of cochlear fibres and because of this, the threshold of detection of the CAP will depend very much on how impulsive the stimulus is. Illustrating this, figure 8.7 shows CAP audiograms determined using stimuli with different envelope rise times. The coarse dashed curve was obtained with tone stimuli having a 1 ms rise time, the solid line with 2 ms rise time, the dashed line with 4 ms rise time, and the dotted line with stimuli having an onset rise time of 10 ms. Clearly, the shorter the rise time, the more impulsive the stimulus will be; the resultant increased synchrony of discharge will make the CAP evoked more obvious and have a lower threshold of detection. GOLDSTEIN & KIANG (1958) demonstrated many years ago this requirement for a stimulus to have a rapid rise time in order to evoke an AP response. Too short a rise time will, however, make the stimulus too broad-band. The trade-off between having an impulsive stimulus, essential for evoking synchronous neural activity, and having a frequency specific

ENERGY SPECTRA OF UNFILTERED TONE PIPS



ENERGY SPECTRA OF 1/3 OCT. FILTERED TONE PIPS

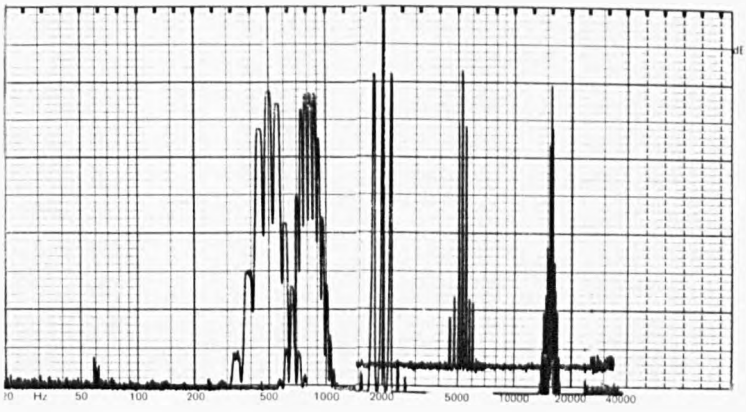


Figure 8.4 A comparison of the energy spectra of a sample of the 4 ms tone pips before and after 1/3 octave band-pass filtering. (The repetition rate of this test signal = 200/s).

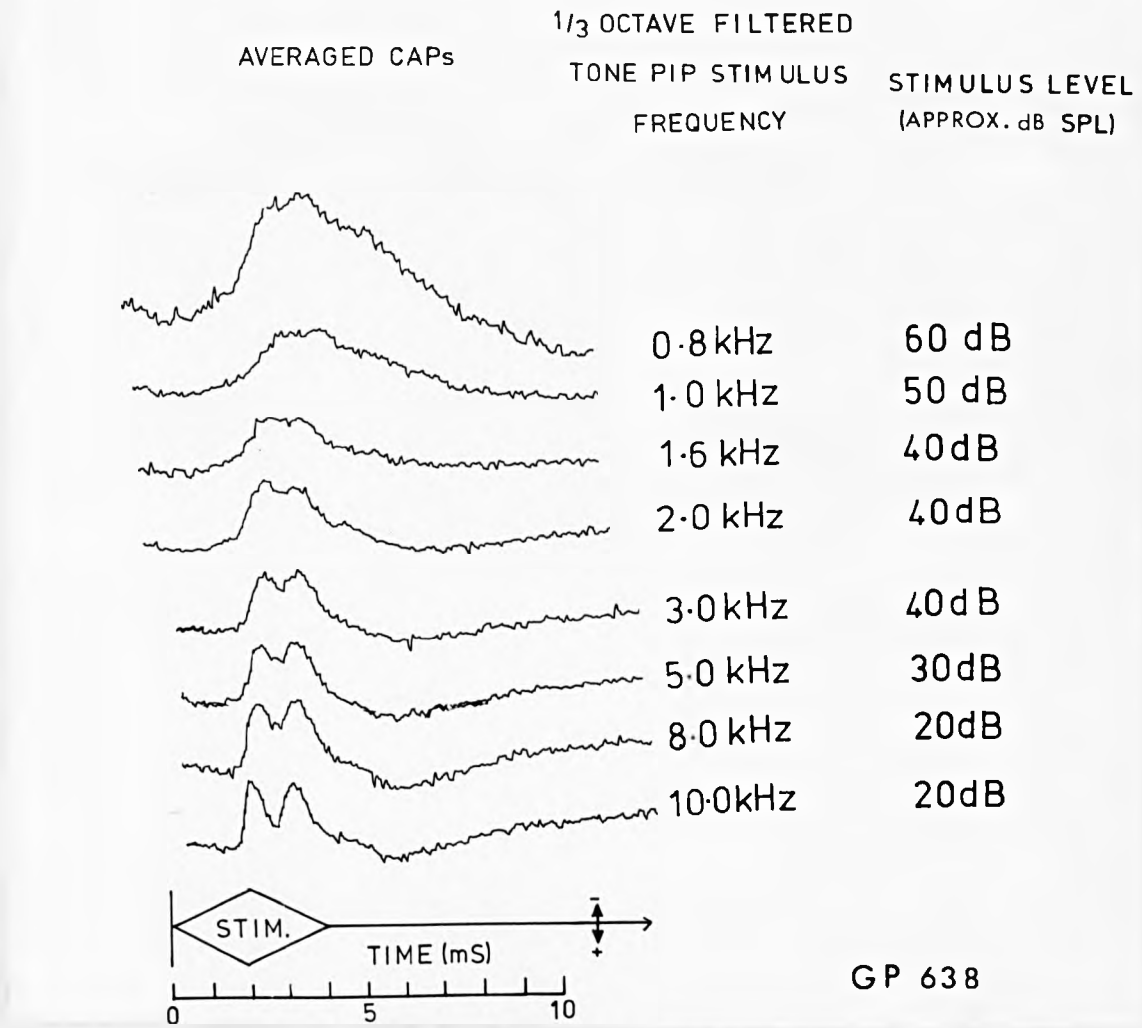


Figure 8.5 Near threshold, averaged CAPs recorded from near GP round window in response to tone pips (2 ms rise fall times) filtered with 1/3 octave band-pass filters. The frequency and intensity of the stimuli are indicated (right). The stimulus delay through the band-pass filter has been corrected for in this figure by shifting the traces appropriately.

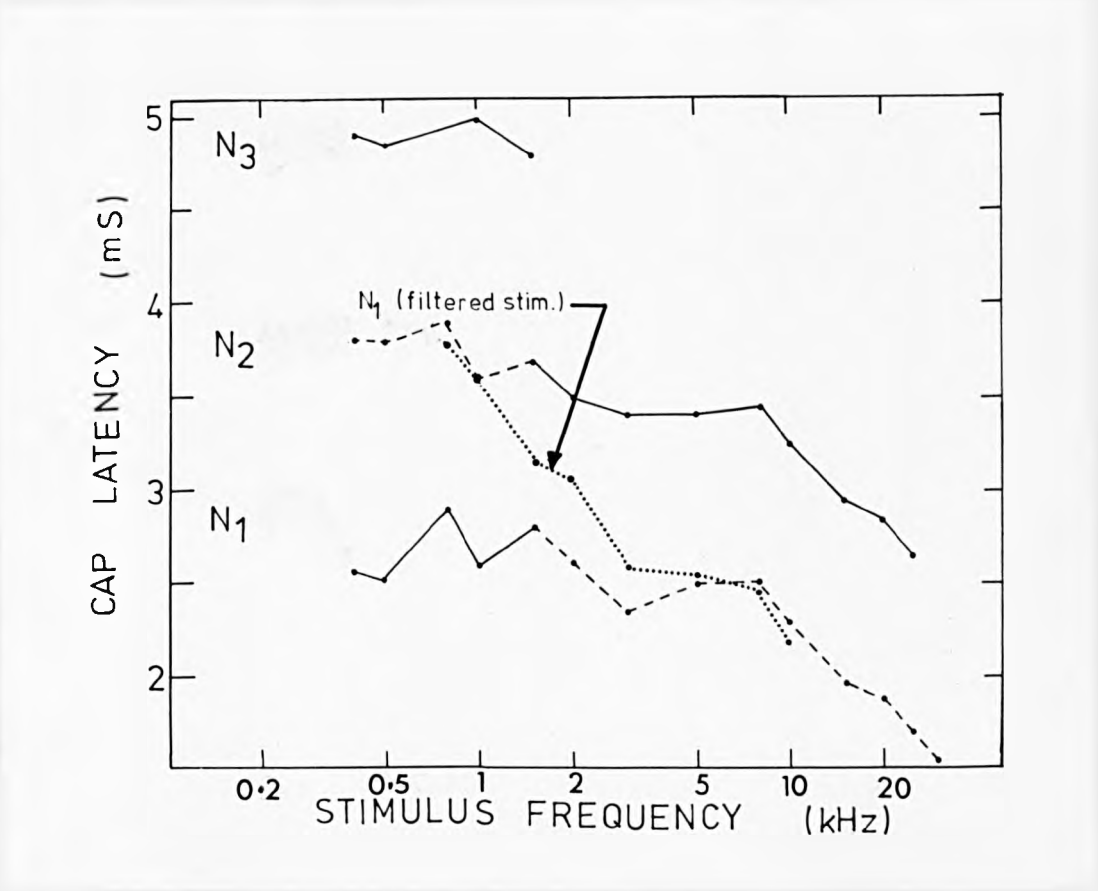


Figure 8.6 The latencies of the N_1, N_2 & N_3 peaks of the averaged CAPs evoked by tone pip stimuli, plotted against the tone pip frequency. The continuous and dashed curves are latencies from CAPs evoked by unfiltered tone pips (from figure 8.3). The dashed curves indicates the peak with the greatest amplitude. The dotted curve is the N_1 latency from CAPs evoked by 1/3 octave filtered tone pips (from figure 8.5).

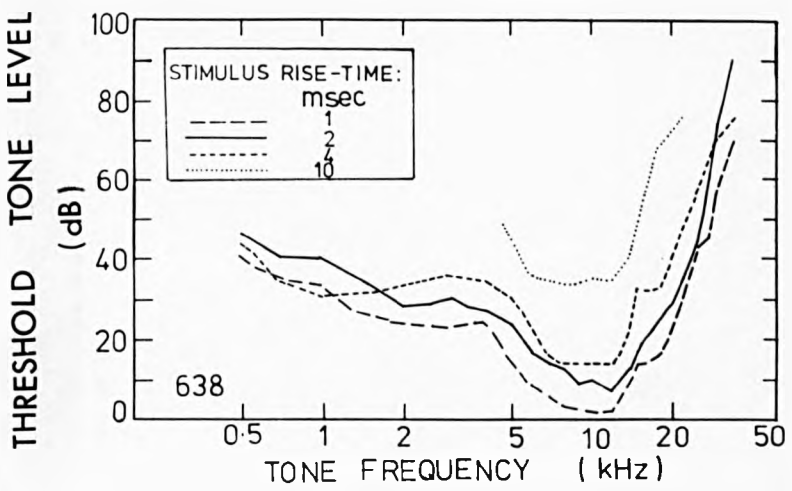


Figure 8.7 CAP audiograms from a normal GP obtained using stimuli with envelope rise times of 1,2,4 and 10 ms (see key). Threshold tone levels represent dB SPL ± 6dB at the tympanic membrane.

stimulus which will excite (near threshold) a particular region of the cochlea is well met by the tone pip used. An even better compromise would be a filtered tone pip (or a pure tone gated by a Gaussian envelope) especially for limiting the spread of energy of low frequency stimuli (see fig. 8.4).

Despite its obvious potential use as a method for objective audiometry, little use has been made of the CAP threshold to tone pip stimuli in either experimental CAP recording or, more importantly, in clinical electrocochleography. ÖZDAMAR et al. (1975) have found that the behavioural threshold and CAP threshold curves (CAP audiograms) of gerbils compare very favourably, both for normal and kanamycin treated animals. They reported that the comparison is good in the frequency range from 500 Hz to 24 kHz. DAVIS (1976) has reported that in clinical electrocochleography, the thresholds of the CAP responses to brief tone pips at 8, 4 & 2 kHz are very close to audiometric thresholds and that 1 kHz tone pips are "also useful". EGGERMONT and coworkers (EGGERMONT & ODENTHAL, 1974, SCHMIDT, EGGERMONT & ODENTHAL 1974) have also found in patients, a good agreement between the subjective threshold, and electrocochleographic threshold to tone pip stimuli.

The present study offers direct evidence that tone pip evoked CAPs can, within the limits discussed, be valid indicators of the threshold of individual cochlear fibres, and hence in cochlear pathology, residual cochlear function.

8.2 THE CAP AMPLITUDE : INTENSITY FUNCTION.

The reasons for investigating amplitude and latency : intensity functions have been stated in the introduction (section 1.1b,) and in the results section 5.2. To reiterate, the main concern was a) with the origin of the form of these functions, particularly those from cochleas with OHC loss, and also b) whether any feature of the function could be a useful indicator of the degree or extent of a cochlear lesion.

CAP amplitude : intensity, functions have been measured extensively in experimental animals, and in human electrocochleography (e.g. cat: DERBYSHIRE & DAVIS, 1935; ROSENBLITH, 1954; PEAKE & KIANG, 1962; RADIONOVA, 1963; STANGE et al. 1964; WIEDERHOLD & KIANG, 1970. GP: TAUB & NAAB, 1969; NIEDER & NIEDER, 1970; SPOOR & EGGERMONT, 1973; BONE et al. 1972; EGGERMONT & SPOOR, 1973; EGGERMONT & ODENTHAL, 1974; DALLOS & WANG, 1974; ARAN & DARROUZET, 1975; ARAN & CHARLET de SAUVAGE, 1975. Rat: CROWLEY et al. 1972. Man: YOSHIE, 1966; YOSHIE & OHASHI, 1969; ARAN, 1971, 1973; SALOMON & ELBERLING, 1971; FORTMANN et al. 1973; EGGERMONT & ODENTHAL, 1974; TYBERGHEIN, 1975; MONTANDON et al. 1975a, b; NAUTON & ZERLIN , 1976; ELBERLING & SALOMON, 1976; ELBERLING, 1976; THORNTON, 1976).

These functions, when plotted with a linear amplitude ordinate and with a stimulus intensity abscissa in dB, are often described as typically having two segments. Over low intensities the amplitude of the CAP response grows slowly, and at higher intensities it increases rapidly. YOSHIE (1966) described these segments as L & H curves respectively. (The 'two segment' description has even been forced upon functions which show no obvious two segments e.g. ARAN 1973). This 'typical' shape to the amplitude : intensity function has commonly been explained in terms of different populations of nerve fibres or receptors which are predominantly active at either low or

high intensities of stimulation (e.g. DAVIS et al. 1958; YOSHIE, 1968; ROSENBLITH, 1954; RADIONOVA, 1963; YOSHIE & OHASHI, 1969; STANGE et al. 1964; KEIDEL, 1970; ARAN, 1971; PORTMANN et al. 1973; EGGERMONT & ODENTHAL, 1974). This explanation is untenable because a) there is no good evidence, anatomical or physiological, for more than one population of cochlear fibres (see section 1.3d for details), and b) the two segments are not an invariant feature of such functions. This is clearly demonstrated by the click evoked amplitude : intensity functions of the present study (figure 5.12). The amplitude of the CAP increases linearly with increasing stimulus intensity in dB. On the other hand, functions obtained with frequency specific stimuli (figure 5.13) show the 'classical' two segments. A survey of published amplitude : intensity functions (figures 8.8 & 8.9) also indicates that functions obtained with frequency specific stimuli can more easily be described as having a two segment curve than those obtained with more broad band stimuli. (In figures 8.8 & 8.9, functions obtained with frequency specific stimuli are drawn on the right hand side of each figure.) Without details of the energy spectra of the stimuli used (e.g. how broadband the click stimuli actually are) the comparison can only be cursory. Some of the functions obtained with click stimuli do show an inflexion (e.g. top left diagram of figure 8.9) but the important point to note is that it is much less obvious than in functions obtained with frequency specific stimuli. This feature has not previously been explicity emphasized.

An explanation, more convincing than the two population hypothesis, to explain the two segment curve obtained with a frequency specific stimulus has been proposed by EVANS (1975b, c). ÖZDAMAR & DALLOS (1976) proposed a similar explanation. Figure 8.10 (from EVANS 1975b) shows, diagrammatically, the effect of increasing the intensity of a tone stimulus (indicated by the dashed line at T) on the number of cochlear fibres activated. At low intensity in the normal cochlea (top left), as the stimulus intensity increases it will stimulate relatively few fibres. When the stimulus is intense enough to reach the region where the tails of many FTCs overlap at any specific frequency, it will cause many fibres to be active. The number of fibres active, as a function of stimulus intensity, is represented in the lower left-hand diagram. There is a similarity between this plot and the amplitude : intensity function to a tone stimulus, but as EVANS emphasises (1975b), the CAP is the result of a complex interaction of potentials and it is unwise to go too far with a simplistic explanation of gross CAP phenomena. Nevertheless, for a pure tone stimulus, EVANS' hypothesis goes some way to explain

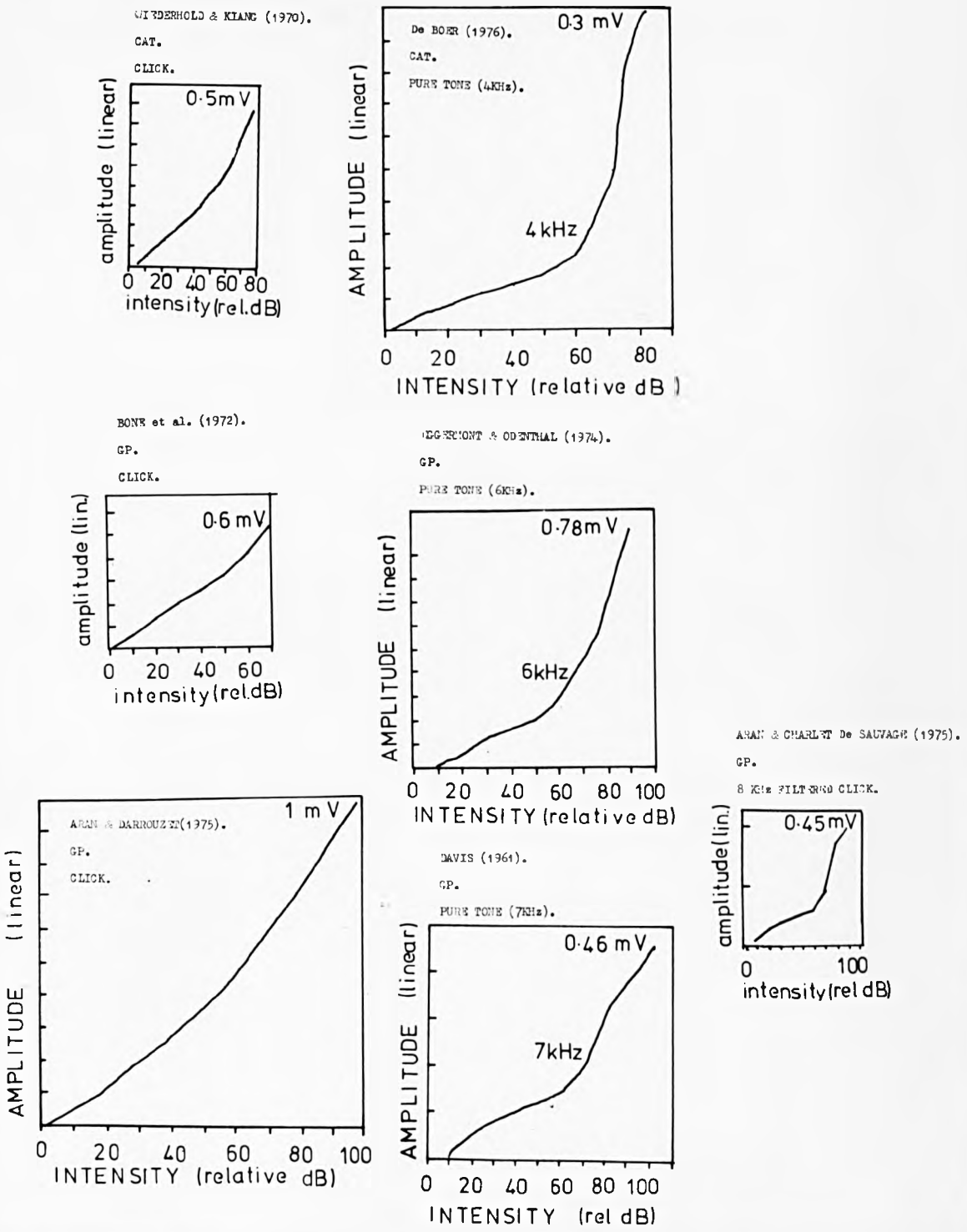


Figure 8.8 CAP amplitude (linear scale) versus intensity (dB) functions for cat and GP, redrawn from the literature. The species and stimulus used are indicated on each diagram. The functions to the left of the figure were determined with broadband (click) stimuli while those on the right were determined using frequency specific stimuli.

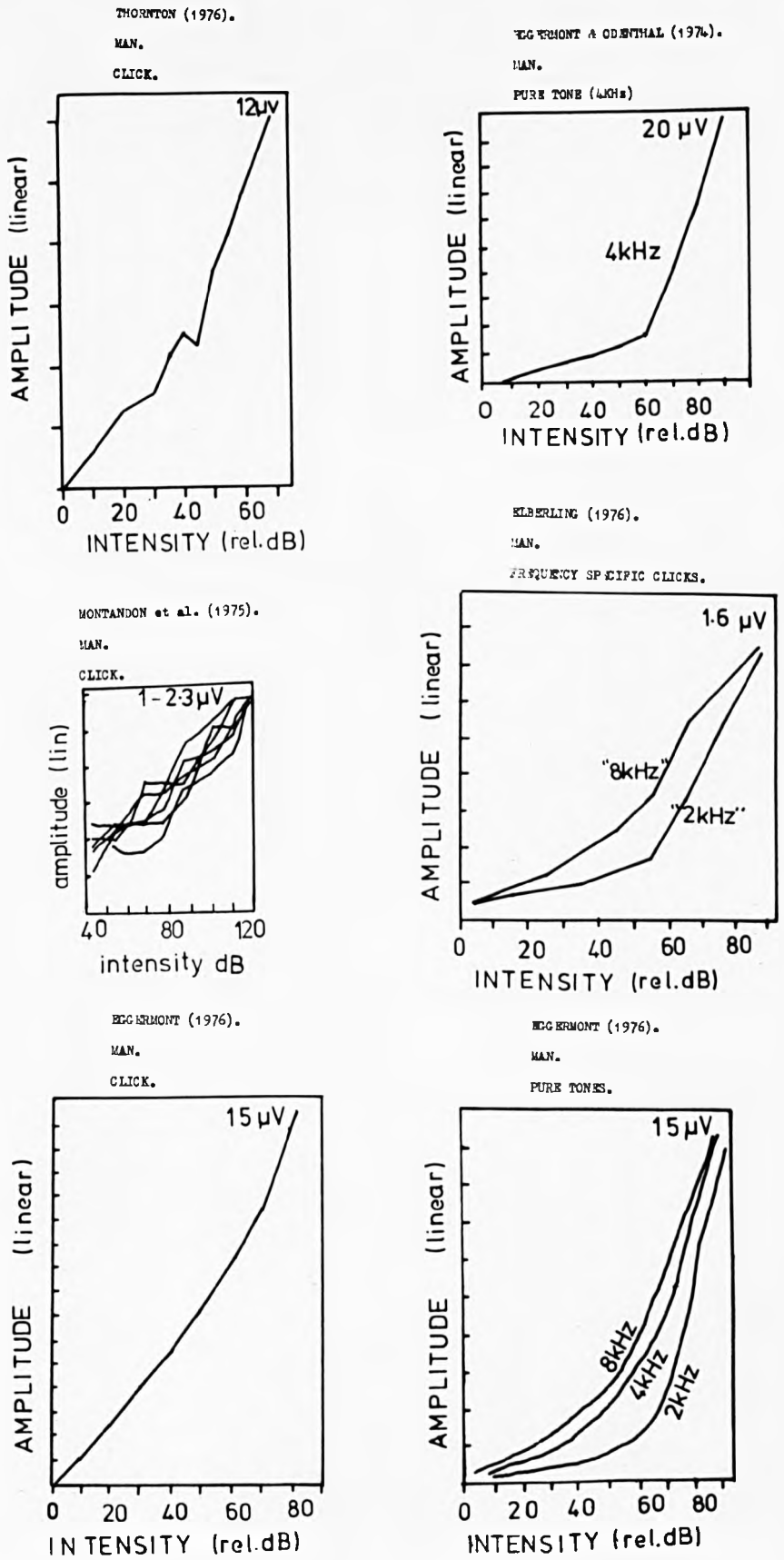


Figure 8.9 CAP amplitude (linear scale) versus intensity (dB) functions for man, redrawn from the literature. The stimulus used is indicated on each diagram. The functions to the left of the figure were determined with broad-band click stimuli, while those on the right were determined using frequency specific stimuli.

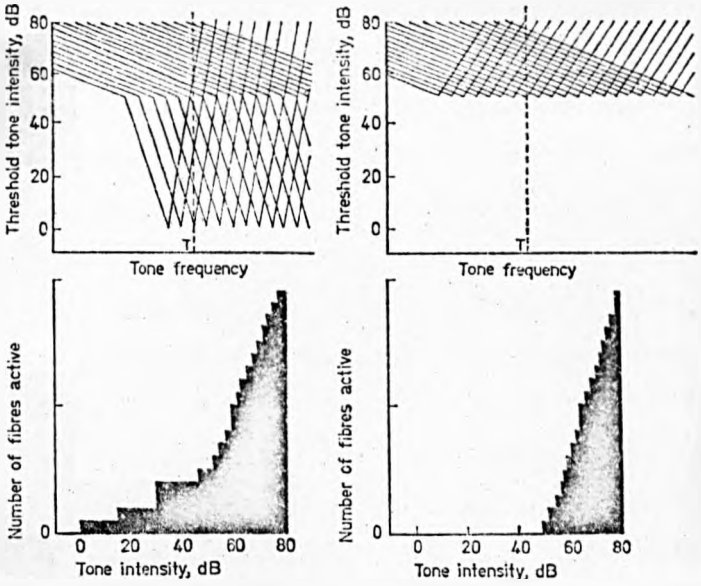


Figure 8.10 (from EVANS 1975b). A schematic diagram illustrating, hypothetically, the growth of the number of active cochlear fibres with increasing tone stimulus level, in the normal cochlea (left diagrams) and a pathological cochlea (right diagrams). The tone is indicated by the dashed line at T. The growth in the number of active fibres with increasing tone level is represented in the lower graphs. A fibre is added to the active group as the tone level crosses its FTC. The scales in each plot are arbitrary. The growth of the number of active fibres may relate to the growth in amplitude of the gross cochlear action potential with increasing stimulus intensity.

the two segment amplitude: intensity function.

De BOER (1975) has attempted to model the amplitude : intensity function more formally, using known properties of cochlear fibre responses, particularly their frequency selectivity, and rate function (i.e. the way the firing probability of a nerve fibre depends upon the intensity of the stimulus). In this model, the CAP waveform has been synthesized from assumed unit functions (the signal wave form that every firing of each fibre contributes to the CAP). De BOER has compared theoretical amplitude: intensity functions produced from his model to experimental functions from the normal cat (using analogous tone pip stimulus parameters), and found a discrepancy: "the theory fails to explain the observed extra rise of the CAP amplitude for stimulus intensities above 70 dB S.L". One contributing reason for this was that the hypothetical frequency selectivity of cochlear fibres used in the model were derived from impulse responses obtained by reverse correlation, and unlike the proposals of EVANS and of ÖZDAMAR did not include tails to the tuning curves. It is clear that their inclusion would go some way towards explaining the discrepancy in the model. De BOER (1975) acknowledges this, but doubts whether it could fully account for the increase in CAP amplitude at high intensity.

EVANS' hypothesis (and that of ÖZDAMAR & DALLOS) also fails to account for the rise in CAP amplitude at high stimulus intensities, if broad band stimulation is considered. This is illustrated in figure 8.11, which diagramatically shows the region of the high frequency cut-off of the audiogram. (The actual slope of the cut-off is not important.) The broad band stimulus (click) is represented as having a flat energy spectrum. The number of fibres active at increasing stimulus intensity is shown in the lower graph. The hypothetical CAP amplitude would increase rapidly at low intensities and much less rapidly at high intensity. The slope of the function at high stimulus intensities, predicted by this simple extension to EVANS' hypothesis, is clearly not adequate to explain the slope of the normal amplitude function to broadband stimuli (e.g. as shown in the results of the present study in figure 5.12).

In the models of EVANS, De BOER, and ÖZDAMAR & DALLOS, excitation spreads to high frequency regions of the cochlea as the stimulus intensity is increased. Derived CAP studies (CAPs representing action potentials originating from frequency bands alongthe cochlea) indicate that the CAPs produced from the high frequency region of the cochlea have larger amplitude than those from more apical regions (TEAS et al. 1962; EBELING, 197[illegible]; EGGERMONT & ODENTHAL, 1974). Thus it is to be expected that a basalward spread of excitation would produce an increase in CAP amplitude.

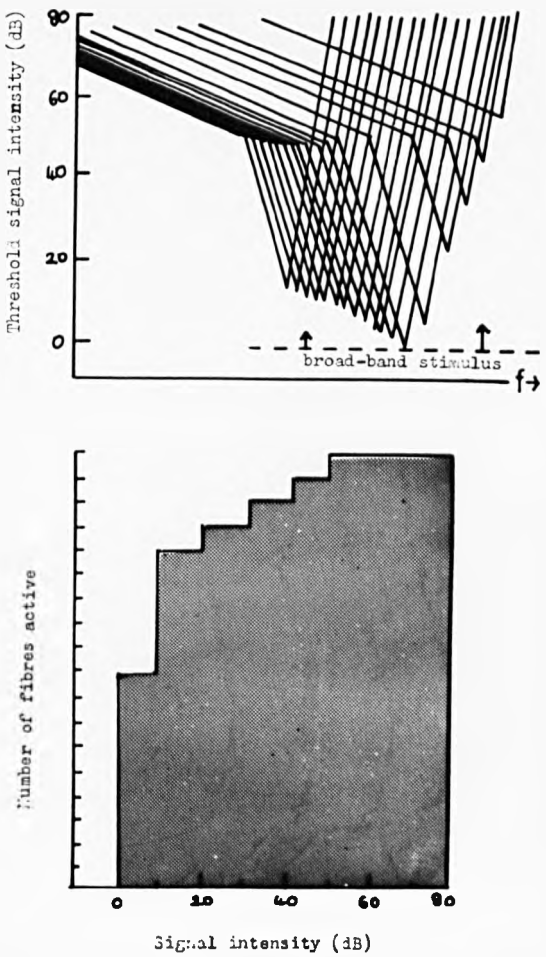


Figure 8.11 Schematic diagram to demonstrate the increase in the number of a cochlear fibres activated as the intensity of a broad band click stimulus is increased. (compare with figure 8.10).

In the upper diagram, stylized FTCs of the HF cut-off of the audiogram are shown. The number of fibres activated by the stimulus as its intensity is increased is represented in the lower graph.

De BOER (1977) suggested that the increase in amplitude of the CAP is because the contribution from the basal neurones in the cochlea are 10-20 times larger than those of more apically located fibres i.e. the unit functions of fibres in the basal turn are larger than those in other turns. It is possible that the site of origin of basal fibre unit functions is closer to a roundwindow electrode than the origin of unit functions from more apical cochlear fibres. However, the rise in the CAP amplitude function at high stimulus intensities is also found when the recording electrode is essentially equidistant from all parts of the cochlea (e.g. ear canal recordings).

Some authors regard the larger CAP amplitude from basal fibres to result primarily from the greater synchrony of discharge in such a fibre population (e.g. DEATHERAGE et al. 1959; TEAS et al. 1962; ELBERLING, 1976). The inclusion of a weighting function, to account for changes in the synchrony of fibre discharge with frequency, would improve (at least qualitatively) the suggestion of EVANS, and the model of ÖZDAMAR for the CAP amplitude : intensity function for click stimuli.

8.3 CAP AMPLITUDE : INTENSITY FUNCTIONS IN COCHLEAR PATHOLOGY.

The results of the present study (figures 5.14-5.18) have shown that the hair cell loss caused by kanamycin poisoning (and therefore loss of the low threshold and sharply tuned segment of cochlear fibre FTCs) has raised the threshold of the CAP response, and reduced its amplitude in response to maximum stimulus intensities.[1] The elevated threshold corresponds to the elevation of the minimum thresholds of cochlear fibres. The decrease in maximum amplitude is most likely to be the result of loss of IHCs (and OHCs) in the extreme basal region of the cochlea as a result of kanamycin poisoning. Thus, fibres from such regions, which would normally be active at high stimulus intensities, no longer contribute to the CAP. This will be the case for broadband stimuli, and for tone pips when such stimuli fall within the tails of the FTCs of high CF fibres. Because the most basal fibres contribute most to the CAP (e.g. because of their synchronous discharge), small areas of inactive or missing fibres could result in a large decrease in the maximum amplitude of the CAP compared to the maximum amplitude in the normal animal.

The plateau in the amplitude : intensity function to tone pip stimuli, often found in the present study (e.g. figure 5.14-5.17), is possibly the result of a 'saturation' of the most basal fibre population caused when the tone pip stimulus is intense enough to fall within the tail segments of the most basal fibres response areas; a further increase in stimulus intensity would not cause an increase in the number of fibres stimulated.

---

[1] Similar findings have been made by DAVIS et al. (1958), ARAN & DARROUZET, (1975) and ARAN & CHARLET de SAUVAGE (1975), but the cochlear lesions (produced by aminoglycoside poisoning) were not well defined.

It is appropriate to comment on the putative relationship between the steepness of the CAP amplitude : intensity function and loudness recruitment. Steep CAP amplitude : intensity functions with high thresholds have been commonly recorded in clinical electrocochleography (ARAN 1971, 1973; PORTMANN et al. 1973; ELBERLING 1974; ELBERLING & SALOMON 1976) and have commonly been termed recruiting functions. Such functions have often found in patients with loudness recruitment, and some authors (e.g. ARAN, 1973) claim that loudness recruitment can be predicted from the CAP amplitude function. It has been suggested (e.g. EVANS, 1972, 1975b) that loudness recruitment may be associated with the broadening of cochlear fibre FTCs in cochlear pathology. Thus, in figure 8.10 (from EVANS, 1975b) the lower right hand graph illustrates the rapid increase in the number of fibres active as a tone stimulus falls into the low frequency tail region of broadly tuned FTCs. EVANS (1975b) suggested that the steepness of the slope in that graph may bear some relation to the growth of loudness, and may also explain the 'recruiting type' of CAP amplitude : intensity function found in cochlear pathology. It is not relevant to discuss how the loudness percept is related to cochlear fibre activity, and whether, in that respect, EVANS suggestion is tenable. However, the results of the present study do bear upon how the steepness of the cochlear action potential amplitude : intensity function, recorded from pathological cochleas, relates to the increase in fibre activity. For a given threshold elevation, the steepness of the amplitude : intensity function depends entirely on the maximum CAP amplitude. For click stimuli, this maximum amplitude appears to be unrelated to the number of fibres active. Thus in figure 5.18, cochleas 551, 546, 566, 572 & 568, have very similar cochlear lesions (and, by inference, similar deficits of active fibres) but their maximum CAP amplitudes, and in consequence the steepness of the amplitude : intensity functions are very different.[2] Perhaps it is not surprising that MONTANDON et al.[3] (1975) find that this function for patients with recruiting sensorineural hearing loss can be as steep as those from patients with non recruiting hearing losses.

For tone pip stimuli, the steepness of the amplitude : intensity function in cochleas with extensive OHC loss do approach the steepness of the normal function at similar intensities. This could be considered as being compatable with EVANS (1975b,c) hypothesis for the steep slope of pathological amplitude : intensity functions. That is, that the overlapping (broadly tuned high threshold) FTCs of pathological cochlear fibres and the high threshold overlapping tail segments of normal FTCs provide the same substrate for a rapid spread of activity to many fibres as the stimulus intensity is increased. In this respect, the model may be

---

2 It is worth noting that these maximum CAP amplitudes are being compared in absolute terms with the normal maximum amplitude values. If the CAP amplitude: intensity function is plotted as a % of its own maximum, as in figures 5.14-5.16, the slope will always appear steep and of the 'recruiting type'.

3 Using a broad-band click stimulus.

of response to click stimuli occur with increasing intensity of stimulation (e.g. in high CF cochlear fibres (cat) at least 0.5 ms for a stimulus intensity change of 40 dB; KIANG et al. 1965).

As the intensity of the click stimulus is increased, cochlear fibres both apical and basal to these responding at threshold will be stimulated, but on the principle that the most basal of these fibres discharge most synchronously (and perhaps contribute larger unit responses to the CAP) the N_1 latency will be essentially determined by the location along the cochlea of the most basal fibres in any group stimulated. Thus, cochlear fibres whose minimum thresholds form the HF cut-off slope of the audiogram will determine the N_1 latency. At maximum intensity, the latency will be that of the discharge of the most basal fibres. The pattern of latency change is evident in the data of fig. 5.19 shows the latency : intensity functions before and after cochlear hypoxia. The most sensitive (low threshold) part of the audiogram is around 10 kHz both before and after the elevation of thresholds due to cochlear hypoxia. Correspondingly, the N_1 latency at threshold before and after is approximately the same value of 1.5 ms. At high intensities, where the high frequency cut-off of the CAP audiograms (upper diagram) converge, so too at these stimulus intensities, do the latency values.

The same qualitative interpretation can be given for latency functions from grossly pathological cochleas such as those of figures 5.15 & 5.16. Here, because of high frequency threshold elevation (reflected in the CAP audiogram), the most sensitive area of each audiogram is at low frequencies, distant from the basal turn. Correspondingly, at threshold, the latency of the CAP is abnormally long, approaching 2.0 ms. Very little increase in intensity will stimulate the high threshold, basal region, and accordingly the latency jumps to a short value at high intensities. This type of latency jump is often reported in clinical electrocochleography and termed a 'dissociated response'. It is typical of sensorineural hearing loss involving high frequency threshold elevation (AZAR, 1973).

This interpretation of the latency: intensity function is sometimes used for the interpretation of clinical electrocochleographic data (Y. CAZALS (Bordeaux group) personal communication; EGGERMONT, 1976). The principle is also a feature of de BOER's model of CAP amplitude and latency : intensity functions. Thus de BOER (1975) states "...we can understand how the latency of the maximum value of the A P will be mainly controlled by those neural units that are on the border of the excitation pattern towards the region of higher resonance frequencies". The present study has empirically confirmed the general validity of such an interpretation.

compatible with the recent clinical studies by EGGERMONT (1976) which indicate that the steepness of the tone evoked amplitude : intensity function can be a good indicator of loudness recruitment.

EVANS (1975b) suggestion for recruitment also incorporates the possibility that cochlear fibre rate:intensity functions may be abnormally steep in cochlear pathology, and that this may contribute to the loudness recruitment phenomenon and to the rapid growth of the CAP amplitude : intensity function under conditions of cochlear pathology. Again, it is not relevant to comment on the relationship between rate of activity in cochlear fibres and loudness. With regard to the CAP amplitude, it is unlikely that the steepness of the rate function per se will have any influence on the amplitude of the CAP. The first (and usually largest) peak of the CAP is typically of 1 ms in duration, and it is therefore unlikely that more than one action potential from any fibre contributes to it. However, EVANS assumes (personal communication) that the rate : intensity function of a fibre is related to its probability : intensity function (i.e. the probability of a fibre discharge occuring with a certain time period as a function of stimulus intensity). If this relationship holds for the onset response of a fibres discharge, then a population of fibres with steep rate/probability functions will certainly contribute to a rapid increase in CAP amplitude with intensity.

8.4 CAP LATENCY : INTENSITY FUNCTIONS.

CAP latency : intensity functions were measured for both tone pip and click evoked responses, but as stated previously, the latency functions for the tone pip stimulation cannot be determined because of the slow rise time (2 ms) of the stimulus, and are thus not considered.

The click evoked CAP latency changes with intensity in a rather predictable manner. In the case of a broadband stimulus with a relatively flat energy spectrum, the most sensitive part of the audiogram is stimulated at CAP threshold. This seems reasonable on intuitive grounds. The latency of the N_1 response to a click stimulus at threshold in the normal GP is approximately 1.5 ms. (In the eight examples in figure 5.12, these latencies are 1.35- 1.55ms, average 1.49). This value does not compare favourably with the available data on the latency of GP cochlear fibre responses to click stimuli (EVANS 1972; review, EVANS 1975a) which indicate that cochlear fibres in the most sensitive 8 - 10 kHz region have much shorter latencies of 1.0 - 1.5 ms (average 1.3 ms). However, a straight comparison of these two latency values is unreasonable. The N_1 latency represents cochlear fibre activity near threshold, whereas the available data of EVANS is ascertained at sound levels 20-30 dB above minimum threshold. Considerable changes in latency

However, this simplistic interpretation of CAP latency will almost certainly be compounded by latency changes which could occur as a result of changes in the filtering properties of cochlear fibres in cochlear pathology It may be significant in this respect that two of the control cochleas of figure 5.12 (GPs 543 & 613), in which a slight elevation of CAP thresholds occurred (indicating some acute cochlear deterioration) also had latency values at maximum stimulus intensities which were shorter than the other controls. This could have been because the high CF fibres suffered a deterioration in their tuning during the cochlear insult, resulting in a reduction of their filter response times.

One of the purposes of clinical electrocochleography is to assess the pattern and extent of a cochlear lesion, that is, to obtain an objective audiogram. In this respect, the latency : intensity function obtained with a broad band click stimulus is of some value for indicating the high frequency cut-off slope of the audiogram, and therefore the extent of a basal cochlear lesion.

APPENDIX A. Early psychophysical studies indicating a deterioration in frequency selectivity to be associated with sensorineural hearing loss of cochlear origin.

Some early evidence concerning a deterioration of frequency selectivity as a result of cochlear pathology came from the use of noise audiometry, and the measurement of tone thresholds alone, and in the presence of noise masking. These measurements used to obtain the critical ratio i.e. the difference in dB between the threshold, in noise, of a pure tone and the spectrum level of the wide band noise required to mask the pure tone. The critical ratio bears a proportional relationship to the critical bandwidth, i.e. the critical bandwidth is approximately equal to 2.5 x critical ratio. (review: SCHARF, 1970).

ZANGMEISTER (1951) measured the threshold of a pure tone in the presence of different noise levels and found that in at least one case of sensorineural deafness (Ménières syndrome) the ratio of noise to signal had to be lower than in normal subjects (i.e. the critical ratio was larger). HUIZING (1952) used a similar technique, and found that for patients exhibiting recruitment, presumably indicating deafness of the cochlear origin, there was an increase in the critical ratio. He also found that spondees (two part words with equal emphasis on both parts e.g. book-case, back-fire) were masked by lower levels of noise than in normal. LANGENBECK (1953, 1965) measured the pure tone audiogram in the presence of various noise levels and he also found subjects in which their masked threshold was much greater than normal. LANGENBECK, however, did not associate the increase in critical ratio with cochlear impairment, but with neural damage.

PALGOV & TERESCHENKO (1973) reached a similar conclusion to that of LANGENBECK. They demonstrated an increase in the critical ratio (measured as a change in 'efficiency of the critical band') in patients with 'perception deafness'. They associated the deterioration in 'critical band' with neural (including central) pathology. They also investigated the relationship between hearing loss and deterioration of the 'efficiency of the critical band' (critical ratio) and showed that for hearing losses of up to 50 dB, there was no significant change in critical ratio; only higher degrees of hearing loss involved a deterioration in critical ratio.

GUNDERSEN (1958) mentioned a patient with poorer discrimination of speech in noise than for normal subjects and noted that "signs of cochlear changes were found in this ear".

Although there were some contrary opinions concerning the usefulness of the critical ratio as an indicator of deafness of cochlear origin (e.g.

PALVA et al. 1953), it was clear that some patients with sensorineural deafness of cochlear origin did exhibit an increased critical ratio and thus a widening of the critical band.

Further support for the view that a deterioration in frequency selectivity was the result of cochlear pathology came from studies which noted a greater spread of masking by narrow bands of noise, above and below the noise band. (JERGER et al. 1960; RITTMANIC, 1962). Similar findings were reflected in the threshold of octave masking test (TOM) (CLACK & BESS 1969, GRIM & BESS 1973) in which the masking, an octave above the masker, was greater in sensorineural hearing impaired patients than in normal subjects. The results of the last mentioned study were interpreted as being caused by an increase in 2nd harmonic distortion of the input signal. Thus more noise would occur one octave above the masker due to the harmonics of the masker, and furthermore the test tone itself would have less energy because of its own distortion As a consequence the masked tone threshold would be raised. These results however, seem equally suitably explained by assuming that the frequency selectivity by the auditory system has deteriorated.

De BOER & BOUMEESTER (1974), using a simple masking threshold tracking procedure with a notch filtered noise band, found some (but not all) sensorineural deaf patients in which "the critical band mechanism is hardly functioning at all". These authors however seem to have changed their minds about the interpretation of the data in terms of widening of critical bands, and later de BOER & BOUMEESTER (1975) preferred to think in terms of pronounced asymmetry of masking (PAM) caused as a result of harmonic distortion, again however, the PAM could be explained by a deterioration of auditory filtering (see note added in proof to de BOER & BOUMEESTER 1975 based on comment by E.F. EVANS).

More direct measures of critical band have shown that a deterioration in frequency selectivity occurs in sensorineural deafness. De BOER (1961) gave an example of a recruiting ear with an increased critical band determined by the technique of measuring masked thresholds for narrow bands of noise of increasing bandwidth (spectral density of noise is constant). The bandwidth at which some of the energy of the masking noise starts to fall outside the critical band (hence not contributing to masking) gives the value for the critical bandwidth. Using another technique - loudness summation, SCHARF & HELLMAN (1960) and BONDING (1976) showed greater critical bandwidths in subjects having cochlear deafness.

MARTIN (1974) measured, in cochlear deaf patients, the loudness of pairs of tones with various frequency spacings (using a loudness balancing technique). His results suggest "extensive widening of the critical band mechanism".

ANDERSON, D.J., ROSE, J.E, HIND, J.E., & BRUGGE, J.F. (1971): Temporal position of discharges in single auditory nerve fibers within the cycle of a sine-wave stimulus: frequency and intensity effects. J. Acoust. Soc. Amer. 49, 1131-1139.

ANDERSON, H, & WEDENBURG, E. (1965): A new method for hearing tests in guinea pig. Acta otolaryngol. (Stockh.) 60, 375-393.

ANNIKO, M. (1976): The cytocochleogram in Atoxyl-treated guinea pigs. Acta Otolaryngol. 82, 70-81.

ARAN, J.M. (1971): The electrocochleogram: Recent results in children and some pathological cases. Arch. Ohr. Nas. Kehlkheik. 198, 128-143.

ARAN, J.M. (1973): Clinical measures of 8th nerve function. Advances in Oto-Rhino-Laryng. Karger: Basel, 20, 374-394.

ARAN, J.M., & CHARLET de SAUVAGE, R. (1975): Normal and pathological adaptation of compound 8th nerve responses in the guinea pig. Acta Otolaryng. 79, 259-265.

ARAN, J.M., & DARROUZET, J. (1975): Observation of click evoked compound 8th nerve responses before, during and over seven months after kanamycin treatment in G.P. Acta Otolaryngol. 79, 24-32.

AUDOUIN, P., SAFT, L, & JOBARD, P. (1963): Étude electrophysiologique et histologique de l' ototoxicité de certains antibiotiques. Acta Oto-Laryng. 56, 106-112.

ARTHUR, R.M., PFEIFFER, R.R., & SUGA, N. (1971): Properties of "two-tone inhibition" in primary auditory neurones. J. Physiol. 212, 593-609.

BALOGH, K., HIRAIDE, F. & ISHII, D. (1970): Distribution of radio-active dihydrostreptomycin in the cochlea. An autoradiographic study. Ann. Otol. Rhinol. Lar. 79, 641-652.

BEAGLEY, H.A. (1965): Acoustic trauma in guinea pigs I Electrophysiology and histology. Acta Otolaryng. 60, 437-451.

BECK, C., & KRAHL, P. (1962): Experimentelle und Feingeweblich Unter suchungen über die Ototoxicität von kanamycin. Arch. Ohr. Nas. Kehlkopheilk. 179, 594-610.

BÉKÉSY, G. von (1944): Über die mechanische Frequenzanalyze in der Schnecke verschiedener Tiere. Akust. Zeits. 9, 3-11.

BÉKÉSY, G. von. (1953): Description of some mechanical properties of the organ of Corti. J. Acoust. Soc. Amer. 25, 786-790.

BÉKÉSY, G. von. (1960): Experiments in hearing. McGraw-Hill: N.Y.

BENITEZ, J.T., SCHUKNECHT, H.F. & BRANDENBURG, J.H. (1962): Pathologic changes in human ear after kanamycin. Arch. Otolaryngol. 75, 192-197.

BENNETT, M.V.L. (1970): Comparative physiology: electric organs. Ann. Rev. Physiol. 32, 471-528.

BILLONE, M. & RAYNOR, S. (1973): Transmission of radial shear forces to cochlear hair cells. J. Acoust. Soc. Amer. 54, 1143-1156.

BOER, E. De, (1961): Measurement of the critical bandwidth in cases of perception deafness. In: proceedings of 3rd Int. Cong. Acoust. Elsevier Press: Amsterdam.

BOER, E. De, (1975): Syntnetic whole nerve action potentials for the cat. J. Acoust. Soc. Amer., 58, 1030-1045.

BOER, E. De, (1977): Cochlear events and whole nerve action potentials. Audiology. 16, 380-388.

BOER, E. De, & BOUMEESTER, J. (1974): Critical bands and sensorineural hearing loss. Audiology 13, 236-259.

BOER, E. De, & BOUMEESTER, J. (1975): Clinical psychophysics. Audiology 14, 274-299.

BONACCORSI, P. (1965): Il colore dell'iride come test di valutazione quantitativa, nell'uomo, della concentrazzione di melanina nella stria vascolare. Annali Lar. Otol. Rinol. Faring. 64, 725-738.

BONDING, P. (1976): Critical bands in patients with hearing loss due to salicylate. In: abstracts of 13th Int. Cong. Audiol. Firenze. P142.

BONE, R.C. CROWLEY, D., & RAUCHBACH, E. (1972): 8th nerve round window action potentials masked by high frequency noise in rats and guinea pigs: a comparative study. Laryngoscope. 82, 1499-1513.

BREDBURG, G. (1968): Cellular pattern and nerve supply of the human organ of Corti. Acta Otolaryngol. (Stockh.) Suppl. 236.

BREDBURG, G., & HUNTER-DUVAR, I. M. (1975): Behavioural tests of hearing and inner ear damage. In: Handbook of sensory physiology, volume v/2: auditory system. Springer-Verlag: Berlin. 261-306.

BRUNS, V. (1977): Peripheral auditory tuning for fine frequency analysis by the CF-FM Bat, Rhinolophus ferrumequinum. II Frequency mapping in the cochlea. J. Comp. Physiol. 106, 87-97.

CANNELL, H. (1972): Pentobarbitone sodium anaesthesia for oral and immunological procedures in the guinea pig. Lab. Animals, 6, 55-60.

CARNEY, A.E., & NELSON, D.A. (1976): Psychophysical tuning curves in normal and pathological ears. 92 nd Meet. A.S.A. J. Acoust. Soc. Amer. 60. 5104.

CAPRANICA, R.R. & MOFFAT, A.J.M. (1975): Selectivity of the peripheral auditory system of Spadefoot toad (Scaphiopus couchi) for sounds of biological significance. J. Comp. Physiol. 100, 231-249.

CLACK, T.D., & BESS, F.H. (1969): Aural harmonics: The tone-on-tone masking vs. the best beat method in normal and abnormal listeners. Acta Otolaryngol., 67, 399-412.

CLARK, W.S., CLARK, C.S., MOODY, D.B., & STEBBINS, W.C. (1974): Noise induced hearing loss in chinchilla as determined by + ve reinforcement technique. J. Acoust. Soc. Amer. 50, 1202-1209.

COLEMAN, J.W. (1975): Haircell ratios in the spiral organ of the guinea pig. British Journal of Audiology, 9, 19-23.

CRANDELL, W.E. (1975): Diode pump cochlear audition theory, Int. J. Neuroscience. 6, 203-213.

CROWLEY, D.E., SCHRAMM, V.L., SWAIN, R.E., MAISEL, R.H., RAUCHBACH, E., & SWANSON, S.N. (1972): An animal model for presbyacusis. Larygoscope, 82, 2079-2091.

DALLOS, P. (1969): Combination tone $2f_1 - f_2$ in microphonic potentials. J. Acoust. Soc. Amer. 46, 1437-1444.

DALLOS, P. (1973): Cochlear potentials and cochlear mechanics. In: Basic mechanisms in hearing. Møller. A.R. ed. New York: Academic Press. 335-372.

DALLOS, P., BILLONE, M., DURRANT. J. WANG, C-Y., & RAYNOR, S. (1972): Cochlear inner and outer hair cells: functional differences. Science, 177, 356-358.

DALLOS, P. & CHEATHAM, M.A. (1974): Microphonic correlates of $2f_1 - f_2$. In: Facts and models in hearing (ZWICKER, E. & Terhardt, E. eds) Springer Verlag: Berlin 312-322.

DALLOS, P. & CHEATHAM, M.A. (1976): Production of cochlear potentials by inner and outer hair cells. J. Acoust. Soc. Amer. 60, 510-512.

DALLOS, P. & HARRIS, D. (1977): Inner-outer hair cell interation. In Psychophysics and Physiology of hearing (E.F.Evans and J.P. Wilson eds) Academic Press: London. P 147.

DALLOS, P. & RYAN, A (1975): Frequency selectivity mediated by inner hair cells alone. J. Acoust. Soc. Amer. 57, 540.

DALLOS, P., RYAN, A., HARRIS, D., McGEE, T., & ÖZDAMAR, Ö. (1977): Cochlear frequency selectivity in the presence of hair cell damage. In: Psychophysics and Physiology of hearing Evans, E.F. & Wilson, J.P. eds. London: Academic Press. 249-260.

DALLOS, P. & WANG, C. -Y. (1974): Bio-electric correlates of kanamycin intoxication. Audiology, 1974, 13, 227-289.

DAVIS, H. (1976): Principles of electric response audiometry. Ann. Otol. Rhinol. Laryngol. 28, suppl 28, 3-93.

DAVIS, H., DEATHERAGE, B.H., ROSENBLUT, B., FERNANDEZ, C., KIMURA, R. & SMITH, C.A. (1958): Modifications of cochlear potentials produced by streptomycin poisoning and by venous obstruction. Laryngoscope (St. Louis) 68, 596-627.

DAVIS, H., DWORKIN, S., LURIE, M.H. & KATZMAN, J. (1937): Behavioural, electrical, and anatomical studies of abnormal ears. Laryngoscope, 47, 435-447.

DAYAL, V.S. & BAREK, W.G. (1975): Cochlear changes from noise, kanamycin and ageing. The Laryngoscope 85, 11, 3-18.

DAYAL, V.S., KOKSHANIAN, & MITCHELL, D.P. (1971): Compound effects of noise and kanamycin. Ann. Otol. Rhinol. Lar. 80, 897.

DEATHERAGE, B.H., ELDREDGE, D.H. & DAVIS, H. (1959): Latency of action potentials in the cochlea of GP. J. Acoust. Soc. Amer., 31, 479-486.

DERBYSHIRE, A.J., DAVIS, H. (1935): The action potentials of the auditory nerve. Amer. J. Physiol. 113, 476-504.

DOLAN, T.R., ADES, H.W., BREDBERG, G., & NEFF, W.D. (1975): Inner ear damage and hearing loss after exposure to tones of high intensity. Acta Otolaryngol. 80, 343-352.

DUIFUIS, H. (1972): Thesis: Perceptual analysis of sound. Eindhoven.

DUIFHUIS, H. (1974): An alternative approach to the second filter. In Facts and models in hearing. Springer-Verlag: Berlin. 100-104.

DUIFHUIS, H. (1977): Cochlear non linearity and second filter. A psychophysical evaluation. In: Psychophysics and physiology of hearing. Academic Press: London. 151-163.

EGGERMONT, J.J. (1976): Electrocochleography. In: Handbook of Sensory Physiology. Vol V/3. Springer-Verlag:Berlin. 623-705.

EGGERMONT, J.J. & ODENTHAL, D.W. (1974): Methods in electrocochleography. Acta Otolaryng. Supp 316, 17-24.

EGGERMONT, J.J., & SPOOR, A. (1973): Cochlear adaptation in guinea pigs. Audiology, 12, 193-220.

ELBERLING, C., (1974): Action potentials along the cochlear partition recorded from the ear canal in man. Scand. Aud. 3, 13-19.

ELBERLING, C. (1976): Action potentials recorded from the promontory and the surface compared with recordings from the ear canal in man. Scand. Audiol. 5, 69-78.

ELBERLING, C., & SALOMON, G. (1976): Action potentials from pathological ears compared to potentials generated by a computer model. In: Electrocochleography. University park press: Baltimore. 439-455.

ELDREDGE, D.H., MILLS, J.H., & BOHNE, B.A. (1973): Anatomical, behavioural and electrophysiological observations on chinchillas after long exposure to noise. Advances in Oto-Rhino-Laryng. Vol 20, Karger: Basel. 64-81.

ELLIOTT, D.N., & Mc GEE, T.M. (1965): Effects of cochlear lesions upon audiograms and intensity discrimination in cats. Ann. Otol. Rhinol. Laryngol. 74, 386-393.

ENGSTRÖM, H., ADES, H.N., & ANDERSSON, A. (1966): Structural pattern of the organ of Corti. Stockholm: Almqvist & Winksell.

EVANS, E.F. (1972): Narrow 'tuning' of cochlear nerve fibre responses in the guinea pig. J. Physiol., 206, 14-15P.

EVANS, E.F. (1970b): Narrow 'tuning' of the responses of cochlear nerve fibres emanating from the exposed basilar membrane. J. Physiol., 208, 75-76P.

EVANS, E.F. (1972): The frequency response and other properties of single fibres in the guinea pig cochlear nerve. J. Physiol., 226, 263-287 .

EVANS, E.F. (1974a): The effects of hypoxia on the tuning of single cochlear nerve fibres. J. Physiol., 238, 65-67P.

EVANS, E.F. (1974b): Auditory frequency selectivity and the cochlear nerve. In: Facts and Models in Hearing, 118-129, Zwicker, E. and Terhardt, E. eds. Springer-Verlag: Heidelberg.

EVANS, E.F. (1975a): The cochlear nerve and cochlear nucleus. In: Handbook of Sensory Physiology Vol. V pt. 2, Auditory System. Keidel, W.D. and Neff, W.D. (eds), 1-108. Springer-Verlag. Heidelberg.

EVANS , E.F. (1975b): Normal and abnormal functioning of the cochlear nerve. In: Sound Reception in Mammals: Symp. Zool. Soc. Lond. 197. No. 37. 133-165. Academic Press, London.

EVANS , E.F. (1975c): The sharpening of cochlear frequency selectivity in the normal and abnormal cochlea. Audiol. 14: 419-442.

EVANS , E.F. (1976a): Temporary sensorineural hearing losses and 8th nerve changes. In: Effects of Noise on Hearing: critical issues. 199-221. D. Henderson, R.P. Hamernik, D.S. Dosanjh, and J.H. Mills (eds). Raven Press N.Y.

EVANS , E.F. (1976b): The effective bandwidth of individual cochlear nerve fibres from pathological cochleas in the cat. In: Disorders of Auditory Function 11. Stephens, S.D.G. (ed). 99-110. Academic Press, London.

EVANS , E.F. (1977): Some interactions between physiology and psychophysics in acoustics. Proc. 9th Internat.Cong. on Acoustics, Vol of Invited Review Lectures. 55-65 Madrid.

EVANS, E.F. (1978a): Neuroleptanaesthesia for the GP: an ideal anaesthetic procedure for physiological studies of the auditory system. Arch. oto-laryngol. In press.

EVANS, E.F. (1978b): Single unit studies of mammalian cochlear nerve. In: Technological Basis of Auditory Investigations. H.A.Beagly ed. Oxford university press. In press.

EVANS , E.F. & HARRISON, R.V. (1976): Correlation between outer hair cell damage and deterioration of cochlear nerve tuning properties in the guinea pig, J. Physiol. 256, 43-44P.

EVANS, E.F. & KLINKE, R. (1974): Reversible effects of cyanide and furosemide on the tuning of single cochlear fibres. J. Physiol. 242, 129-131P.

EVANS , E.F. & WILSON, J.P. (1971): Frequency sharpening of the cochlea: the effective bandwidth of cochlear nerve fibres. Proc. 7th Internat. Cong. on Acoustics, 3, Akademiai Kiado, Budapest, 453-456.

EVANS , E.F. & WILSON, J.P. (1973): Frequency selectivity of the cochlea. In: Basic Mechanisims of Hearing. A.R. Moller, ed. Academic Press, N.Y., 519-551.

EVANS, E.F. & WILSON, J.P. (1975): Cochlear tuning properties: concurrent basilar membrane and single nerve fibre measurements. Science, 190: 1218-1221.

FARKASHIDY, J., BLACK, R.G., & BRIANT, T.D.R. (1963): The effects of kanamycin on the internal ear - electrophysiological and electron-microscopic study. Laryngoscope. 73, 713-727.

FATT, P. & KATZ, B. (1951): An analysis of the end-plate potential recorded with an intracellular electrode. J. Physiol. Lond. 115, 320-369.

FLOCK, A. (1965): Electron microscopic and electrophysiological studies on the lateral line canal organ. Acta Otolaryngol. Suppl. 199, 1-90.

FLOCK, Å. (1971): Sensory transduction in hair cells. In: Handbook of sensory physiology Vol 1: Principles of receptor physiology. Springer-Verlag: Berlin. 396-441.

FLOCK, Å. (1977): Physiological properties of sensory hairs in the ear. In: Psychophysics and physiology of hearing (EVANS, E.F. & WILSON, J.P. eds). Academic Press: London. 15-26.

FLOCK, Å. & RUSSELL, I. (1976): Inhibition by efferent nerve fibres: action on hair cells and afferent synaptic transmission in the lateral line canal organ organ of burbot Lota Lota. J. Physiol. 257, 45-62.

FROST, J.O., DALY, J.F. & HAWKINS, J.E.Jr. (1958): The ototoxicity of kanamycin in man. Antibiotics ann. 700-707.

FURMAN, G.G. & FRISHKOPF, L.S. (1974): Model of neural inhibition in the mammalian cochlea. J. Acoust. Soc. Amer. 2194-2201.

FURUKAWA, T., & ISHII, Y. (1967): Neurophysiological studies on hearing in gold fish. J. Neurophysiol. 30, 1377-1403.

FURUKAWA, T., ISHII, Y. & MATSUURA, S. (1972): Synaptic delay and time course of post-synaptic potentials at the junction between hair cells and eight nerve fibres in the goldfish. Jap. J. Physiol. 22, 617-635.

GACEK, R.R. & RASMUSSEN, G.L. (1961): Fiber analysis of the stato-acoustic nerve of guinea pig, cat & monkey. Anat. Rec. 139, 455-463.

GALAMBOS, R. (1956): Suppression of auditory nerve activity by stimulation of efferent fibers to the cochlea. J. Neurophysiol. 19, 424-437.

GÄSSLER, G. (1954): Über die Hörschwelle für schallereignisse mit verschieden breitem Frequenzspektrum. Acoustica, 4, 408.

GEISLER, C. (1974): Hypothesis on the function of the crossed olivocochlear bundle. J. Acoust. Soc. Amer. 56, 1908-1909.

GOLD, T. (1948): Hearing II. The physical basis of the action of the cochlea Proc. Roy. Soc. B. 135, 492-498.

GOLDSTEIN, J.L. (1967): Auditory non-linearity. J Acoust. Soc. Amer. 41, 676-689.

GOLDSTEIN, J.L. (1970): Aural combination tones. In: Frequency analysis and periodicity detection in hearing. (Plomp, R. & Smoorenburg, G.F. eds). Sijthoff: Leiden, 230-247.

GOLDSTEIN, M.H., & KIANG, N.Y-S. (1958): Synchrony of neural activity in electric responses evoked by transient acoustic stimuli. J. Acoust. Soc. Amer. 30, 107-114.

GOLDSTEIN, J.L. & KIANG, N.Y-S. (1968): Neural correlates of the aural combination tone $2f_1 - f_2$. Proc. IEEE, 56, 981-992.

GONZALEZ, G., MILLER, N. & WASILEWSKI, V. (1972): Progressive neuro-ototoxicity of kanamycin Ann. Otol. Rhinol. Lar. 81, 127-131.

GOZOZIK-ZOLNIERKIEWICZ, T. (1969): Outer and inner hair cells in streptomycin poisoning. Laryngoscope. 79, 125-133.

GREEN, C.J. (1975): Neuroleptanalgesic drug combinations in anaesthetic management of small laboratory animals. Lab. Animals, 9, 161-178.

GREENWOOD, D.D. (1961): Auditory masking and the critical band. J. Acoust. Soc. Amer. 33, 484-502.

GRIMM, D.M. & BESS, F.H. (1973): Threshold of octave masking (TOM) test. Acta Otolaryngol., 76, 419-425.

GUILD, S.R., CROWE, S.J., BUNCH, C.C. & POLVOGT, L.M. (1931): Correlation of differencies in the density on innervation of the organ of Corti with differencies in the acuity of hearing. Acta Otolaryng. 15, 209-308.

GUNDERSEN, T. (1958): Paracusis willisii. Acta Otolaryng., 49, 499-508.

GUTH, P.S., HAARSTAD, V.B., & COMIS, S.D. (1974): Staining of hair cell nuclei by a fluorescent derivative of Hemicholinium 3. Acta Otolaryng. 77, 251-255.

HALL, J.G., & RØNNING-ARNESSEN, A. (1974): An electronmicroscopical analysis of the square areas and diameters of the cochlear nerve fibres in cats. Acta Otolaryngol., 77, No 5, 305-310.

HARRISON, R.V., & EVANS E.F. (1977): The effects of hair cell loss (restricted to outer hair cells) on the threshold and tuning properties of cochlear fibres in the guinea pig. In : Colloque Inner Ear Biology (Portmann, M. & Aran, J.-M. eds). Inserm: Paris. 105-124.

HARSPUR, E.S. & D'ARCY, P.F. (1975): The Influence of pigmentation of rats and guinea pigs on the ototoxicity of kanamycin and neomycin. Experientia, 31, 1323-1324.

HAWKINS, J.E.Jr. (1959): The ototoxicity of kanamycin. Ann. Otol. Rhinol. Lar., 68, 698-715.

HAWKINS, J.E.Jr. (1977): Condition of inner hair cells after aminoglycoside intoxication. In: Colloque Inner Ear Biology. (M. Portmann and J.M. Aran eds). Inserm: Paris. 327-334.

HAWKINS, J.E.Jr. & ENGSTRÖM, H. (1964). Effect of kanamycin on cochlear cytoarchitecture. Acta Otolaryngol. Suppl. 188, 100-106.

HAWKINS, J.E.Jr., JOHNSSON, L. -G., STEBBINS, W.C., MOODY, D.B. & COOMBS, S.L.(1976): Hearing loss and cochlear pathology in monkeys after noise exposure. Acta Otolaryngol. 81, 337-343.

HEFTNER, R., HEFTNER, H. & MASTERTON, B. (1971): Behavioural measurements of absolute and frequency difference thresholds in guinea pig. J. Acoust. Soc. Amer. 49, 1888-1895.

HELLE, R.(1974): Enlarged hydromechanical cochlea. Model with basilar membrane and tectorial membrane. In: Facts and models in hearing. Springer: Berlin. 77-85.

HENDERSON, D., HAMERNIK, R.P. & SITLER, R.W. (1974): Audiometric and anatomical correlates of impulse noise exposure. Arch. Otolaryngol. 99, No 1, 62-65.

HENDERSON, D., HAMERNIK, R.P. & SITLER, R.W. (1974): The audiometric and histological correlates of exposure to 1 ms noise impulses in the chinchilla. J. Acoust. Soc. Amer. 56, 1210-1221.

HINSHAW, H.C. & FELDMAN, D.V.M. (1945): Streptomycin in the treatment of clinical tuberculosis, a preliminary report. Proceedings of staff meeting of Mayo Clinic, 20, p313.

HOEKSTRA, A.& RITSMA, R.J. (1977): Perceptive hearing loss and frequency selectivity. In: Psychophysics and physiology of hearing. Academic Press. London. 263-272.

HOOD, J.D., POOLE, J.P. FREEDMAN, L. (1976): The influence of eye colour on TTS. Audiology, 15, 449-464.

HOSHINO, T. (1977): Contact between tectorial membrane and the cochlear sensory hairs in the Human and the monkey. Arch. Oto. Rhino. Laryng. 217, 53-60.

HOUTGAST, T. (1974): Masking patterns and lateral inhibition. In: Facts and Models in hearing. Springer-Verlag: Berlin 258-272.

HOUTGAST, T. (1974): Lateral suppression in hearing. Doctoral dissertation. University of Amsterdam.

HOUTGAST, T. (1977): Phase effects in two tone suppression investigated with a binaural lateralization paradigm. In: Psycho_physics and physiology of hearing. Academic Press: London. 165-170.

HUGGINS, W.H. & LICKLIDER, J.C.R. (1951): Place mechanisms of auditory frequency analysis. J. Acoust. Soc. Am. 23, 290-299.

HUIZING, H.C. (1952): Die Grundsätze der Larmaudiometric.Pract. Oto Rhino Laryngol., 14, 360-366.

HUNTER-DUVAR, I.M. & BREDBERG,G. (1974): Effects of intense auditory stimulation: Hearing losses and inner ear changes in the chinchilla. J. Acoust. Soc. Amer. 55. 795-805.

IGARASHI, M., SCHUKNECHT, H.F., & MYERS, E.N. (1964): Cochlear pathology in humans with stimulation deafness. J. Laryngol. 78, 115-123.

IURATO, S. (1964): Fibre efferenti dinette e crociate alle cellule acustiche del' organo del Corti. Atti. Soc. Ital. Anat. 72, p60.

JERGER, J.F., TILLMAN, T.W., & PETERSON, J.L. (1966): Masking by octave bands of noise in normal and impaired ears. J. Acoust. Soc. Amer. 32, 385-390.

JOHNSTONE, B.M., TAYLOR, K.J. & BOYLE, A.J. (1970): Mechanics of the guinea pig cochlea. J. Acoust. Soc. Amer. 47, 504-509.

JOHNSTONE, J.R. (1977): Properties of ganglion cells from the extreme basal region of guinea pig cochlea. In: Psychophysics and Physiology of Hearing. EVANS, E.F. & WILSON, J.P. eds. London: Academic Press. 89-98.

JØRGENSEN, M.B. & SCHMIDT, M.R. (1962): The ototoxic effect of kanamycin. Acta Otolaryngol. 55, 537-544.

KAKU, J., FARMER, J.C. & HUDSON, W.R. (1973): Ototoxic drug effects on cochlear histochemistry. Arch. Otolaryngol. 98, 282.

KATSUKI, Y., KANNO, A. & SUGA, N. (1961): Primary auditory neurones of the monkey. Jap. J. Physiol. 11. 678-683.

KATSUKI, Y., SUMI, T., UCHIYAMA, H. & WATANABE, T. (1958): Electric responses of auditory neurones in cat to sound stimulation. J. Neurophysiol. 21, 569-588.

KHANNA, S.M., SEARS, R.E.& TONNDORF,J. (1968): Some properties of longitudinal shear waves: a study by computer simulation. J. Acoust. Soc. Amer. 43, 1077-1084.

KIANG, N.Y-S. (1968): A survey of recent developments in the study of auditory physiology. Annals of Otology, 77, 656-676.

KIANG, N.Y-S., LIBERMAN, M.C.& LEVINE, R.A. (1976): Auditory nerve activity in cats exposed to ototoxic drugs and high intensity sounds. Ann. Otol. 85, 752-768.

KIANG, N.Y-S., MOXON, E.C. & LEVINE, R.A. (1970): Auditory nerve activity in cats with normal and abnormal cochleas. In: Sensorineural hearing loss. London. Churchill 241-268.

KIANG, N.Y-S., SACHS, M.B. & PEAKE, N.T. (1967): Shapes of tuning curves for single auditory nerve fibres. J. Acoust. Soc. Amer. 46, 106.

KIANG, N.Y-S., WATANABE, T., THOMAS, E.C., & CLARK, L.F.(1965): Discharge patterns of single fibers in cats auditory nerve. Cambridge Mass: M.I.T. Press.

KIMURA, R.S. (1966): Hairs of the cochlear sensory cells and their attachment to the tectorial membrane. Acta Otolaryngol. 61, 55-72.

KIMURA, R.S. & WERSÄLL, J. (1962): Termination of the olivo-cochlear bundle in relation to the OHCs of the organ of Corti in guinea pig. Acta Otolaryngol. 55, 11-32.

KLINKE, R., & EVANS, E.F. (1977): Evidence that Catecholamines are not the afferent transmitter in the cochlea. Exp. Brain Res. 28. 315-324.

KOHLLÖFFEL, L.U.E. (1971): Studies of the distribution of cochlear potentials along the basilar membrane. Acta Otolaryngol. Suppl. 288.

KOHLLÖFFEL, L.U.E. (1972): A study of basilar membrane vibrations 111. The basilar membrane frequency response curve in the living guinea pig. Acustica, 27, 82-89.

KOHLLÖFFEL, L.U.E. (1973): Observations of the mechanical disturbances along the basilar membrane with laser illumination. In: Basic mechanisms in hearing (Moller, A.R. ed) Academic Press: N.Y. 95-118.

KOHONEN, A. (1965): Effect of some ototoxic drugs on the pattern and innervation of cochlear sensory cells in guinea pigs. Acta Otolaryngol. (Stockh.). Suppl. 208.

KOIDE, Y., HATA, H., & HANDO, R. (1966): Vulnerability of the organ of Corti in poisoning. Acta Otolaryngol. 61, 332-344.

KONISHI, T., TEAS, D.C. & WERNICK, J.S. (1970): Effects of electrical current applied to cochlear partition on discharges in individual auditory nerve fibres. I. Prolonged direct current polarization. J. Acoust. Amer. Soc. 47, 1519-1526.

KROCHMALSKA, E. (1974): The effects of industrial noise & ototoxic antibiotics on cochlear function. Acta Otolaryngol. 77, 47-50.

LA FERRIÈRE, K.A., ARENBERG, I.K., HAWKINGS, J.E.Jr. & JOHNSSON, L.G. (1974): Melanocytes of the vestibular labyrinth and their relationship to the micro-vasculature. Ann. Otol. Rhinol. Lar. 658-694.

LANGENBECK, B. (1953): Diagnosis by testing hearing above threshold. Acta Otolaryngol., 43, 439-465.

LANGENBECK, B. (1965): Text book of Practical audiometry. Arnold: London.

LESHOWITZ, B.H. & LINDSTROM, R. (1977): Measurement of non linearities in listeners with sensorineural hearing loss. In. Psychophysics and physiology of hearing. Academic Press: London. 283-294.

LIDÉN, G. (1953): Acta Otolaryngol. 43, 551-572.

LIM, D.J. (1977): Fine morphology of the tectorial membrane. Fresh and developmental. In. Colloque inner ear Biology. (Portmann M., and Aran, J.M. eds). INSERM: Paris. 47-60.

LINDQUIST, N.G. (1973): Accumulation of drugs on melanin. Acta. Radiol. Suppl. 325, 1-92.

LOWENSTEIN, O. & WERSÄLL, J. (1959): A functional interpretation of the electron microscopic structure of the sensory hairs in the cristae of the elasmobranch Raja elavata in terms of directional sensitivity. Nature (Lond). 184, 1807-1810.

LOWRY, L.D., MAY, M. & PASTORE, P. (1973): Acute histopathologic inner each changes in deafness due to Neomycin: a case report. Ann. Otol. Rhinol. Laryngol. 82, 876-880.

LUNDQUIST, P.G. & WERSÄLL, J. (1966): Kanamycin induced changes in cochlear hair cells of guinea pig. Zeitschirt fur Zell forshung. 72, 543-561.

LUSTBERG, A. & HAMBURGER, M. (1959): 8th nerve deafness after adminstration of kanamycin J.A.M.A. 70, 806.

LYNN, P.A. & SAYERS, B. McA. (1970): Cochlear innervation, signal processing, and their relation to auditory time- intensity effects. J. Acoust. Soc. Amer. 47, 525-533.

MANLEY, G.A. (1977): Frequency dependent extra cellular interaction between hair cells as a possible mechanism for cochlear frequency sharpening. In: Psychophysics and Physiology of hearing. E.F. Evans and J.P. Wilson eds . Academic Press: London 139-146.

MANLEY, G.A. & ROBERTSON, D. (1976): Analysis of spontaneous activity of auditory neurones in the spiral ganglion of the guinea pig cochlea. J. Physiol. 258, 323-336.

MARTIN, M.C. (1974): Critical bands in sensorineural hearing loss. Scandinavian Audiol. 3, 133-140.

MATZ, G.J., WALLACE, T.H., & WARD, P.H. (1965): The ototoxicity of kanamycin. Laryngoscope. (St Louis) 75, 1960-1965.

MATZ, G.J.,BEAL,D.D.,& KRAMES,L. (1969): Ototoxicity of Ethacrynic acid. Arch. Otolaryng. 90, 152-155.

McCELLAND, K.D. & BRANDT J.F. (1969): Pitch of frequency modulated sinusoids. J. Acoust. Soc. Amer. 45, 1469-1498.

McGEE, T., RYAN, A., & DALLOS, P. (1976): Psychophysical tuning curves of chinchilla. J. Acoust. Soc. Amer. 60, 1146-1150.

McGILL, J.J.I., & SCHUKNECHT, H.F. (1976): Human cochlear changes in noise induced hearing loss. Laryngoscope 86, 1293-1303.

MESOLELLA, C. & COSTA, F. (1960): Ototossicita da Kanamycina. Arch. Ital. Otol. 68, 119-152.

MEYER ZUM GOTTESBERGE, A., & STUPP, H. (1969): Streptomycin spiegel in Perilymphe des Menchen. Acta Otolaryng. 67, 171-176.

MONTANDON, P.B., MEGILL, N.D., KAHN, A.R., PEAKE, W.T. & KIANG, N.Y-S. (1975a): Recording auditory nerve potentials as an office procedure. Ann. Otol. 84, 2, 2-10.

MONTANDON, P.B., SHEPARD, N.T., MARR, E.M., PEAKE, W.T., & KIANG, N.Y-S. (1975b): Auditory nerve potentials from ear canals of patients with otologic problems. Ann. Otol. 84, 164-174.

MORRISON, D., SCHINDLER, R.A. & WERSÄLL, J. (1975): A quantitative analysis of the afferent innervation of the organ of Corti in guinea pig. Acta Otolaryngol. 79, 11-23.

NAUTON, R.F. & WARD, P.H. (1959): The ototoxicity of kanamycin sulphate in the presence of compromised neural function: Arch. Otolaryngol. 69, 398.

NAUTON, R.F. & ZERLIN, S. (1976): Human whole nerve responses to clicks of various frequency. Audiology, 15, 1-9.

NEUBERT, K. & WÜSTENFELD, E. (1955): Nachweis der Zellulären anspr-echgebiete im Innenohr. Naturwissen-shaften, 11, 350-351.

NIEDER, P.C. (1974): The cochlea as an opto-acoustical device. Woodhole Press: N.Y.

NIEDER, P. & NIEDER, I. (1970): Antimasking effect of crossed olivo cochlear bundle stimulation with loud clicks in guinea pig. Exp. Neurol, 28, 179-188.

NOMOTO, M. (1977): Responses in auditory fibres to innervation patterns in the cochlea. In: Proceedings of Int. Union. Physiol. Sciences. Vol 13, p553.

ÖZDAMAR, Ö. & DALLOS, P. (1976): Input/output functions of cochlear whole nerve action potentials: interpretation in terms of one population of neurones. J. Acoust. Soc. Amer. 59, No 1, 143-147.

ÖZDAMAR, Ö., DALLOS, P., RYAN, A., & HARRIS, D. (1975): Comparison of auditory thresholds determined behaviourally and electrocochleogr-aphically in normal and kanamycin treated gerbils. J. Acoust. Soc. Amer. 158, suppl. 103p.

PAL'GOV, V.I. & TERESHCHENKO, V.N. (1973): Effectiveness of critical band as indicator of the damage to the auditory system. Biophysics, 18, 773-780.

PALMER, A.R.(1978): PhD dissertation. University of Keele.

PALVA, T., GOODMAN, A., & HIRSH, I.J. (1953): Critical evaluation of noise audiometry. Laryngoscope. 63, 842-860.

PATTERSON, R.D. (1970): Auditory filter shapes derived with noise stimuli. J. Acoust. Soc. Amer. 59, 640-654.

PATTERSON, R.D. (1974): Auditory filter shapes. J. Acoust. Soc. Amer. 55, 802-809.

PEAKE, W.T. & KIANG, N.Y-S. (1962): Cochlear responses to condensation and rarefaction clicks. Biophys. J., 2, 23-34.

PERKINS, R.E. & MOREST, D.K. (1975): A study of cochlear innervation in cats and rats with Golgi methods and Nomarski optics. J. Comp. Neurol. 163, 129-158.

PFEIFFER, R.R. & KIM, D.O. (1972): Response patterns of single cochlear nerve fibres to click stimuli: descriptions for cat. J. Acoust. Soc. Amer. 52, No 6, pt 2, 1669-1677.

PFEIFFER, R.R. (1970): A model for two tone inhibition of single cochlear cochlear nerve fibres. J. Acoust. Soc. Amer. 48, 1373-1378.

PICK, G.F., EVANS, E.F., & WILSON, J.P. (1977): Frequency resolution in patients with hearing loss of cochlear origin. In: psychophysics and physiology of hearing. Academic Press: London 273-281.

PICKLES, J.O. (1975): Normal critical bands in the cat. Acta. Otolarygol. 80, 245-254.

PICKLES, J.O.& COMIS, S.D. (1976): Auditory nerve fibre bandwidths and critical bandwidths in the cat. J. Acoust. Soc. Amer. 60, 1151-1156.

PORTMANN, M., ARAN, J.-M. & LAGOURGUE, P. (1973): Testing for "recruitment" by electrocochleography: preliminary results. Ann. Otol. St. Louis. 82, 36-43.

PRIEDE, V.M. & COLES, R.R.A. (1976): Speech discrimination tests in investigations of sensorineural hearing loss. J. Laryngol. Otol., 90, 1087.

QUANTE, M., STRAUSS, P., & ROSIN, H. (1974): Gentamycin level in the guinea pigs perilymph and liquour subject to the dosis distribution. Arch. Oto. Rhino. Laryng. 208, 295-296.

RADIONOVA, E.A. (1963): Measuring the intensity of a brief sound signal at the first neurone level of the auditory system. Soviet Physics-Acoustics 8, 350-355.

RHODE, W.S. (1971): Observations of the vibration of the basilar membrane in squirrel monkeys using the Mössbauer technique. J. Acoust. Soc. Amer. 49, 1218-1231.

RHODE, W.S. (1973): An investigation of cochlear mechanics using the Mössbauer effects. In: Basic mechanisms in Hearing.Academic Press: N.Y. 49-63.

RHODE, W.S. (1977): Some observations on two tone interaction measured with the Mössbauer effect. In: Psychophysics and Physiology of hearing (E.F. Evans & J.P. Wilson eds). Academic Press: London. 27-39.

RITTMANIC, P.A. (1962): Pure tone masking by narrow-noise bands in normal and impaired ears. J. of. Aud. Res., 2, 287-304.

ROBERTSON, D. (1974): Cochlear neurones: frequency selectivity altered by perilymph removal. Science, 186, 153-155.

ROBERTSON, D. & MANLEY, G.A. (1974): Manipulation of frequency analysis in cochlear ganglion of guinea pig. J. Comp. Physiol., 91, 363-375.

ROESSLER, R. von. (1974): Guinea Pig anaesthesia. Vet. Record 95, 302.

ROMAHN, G. (1974): Influence of kanamycin on the frequency selectivity of cochlear nerve fibres. Proc. 8th Internat. Cong. on Acoustics. 1, 184p.

ROMAHN, G. (1976): Sinneszellspezifische Erregungen auf auditorischen Nervenfasern. Dissertation, Technishe Universitat, Berlin.

ROMAHN, G. & BOERGER, G. (1977): Filter function of guinea pig cochlea after degeneration of outer hair cells. Arch. Oto. Rhino Larng., 215, 223-229.

ROSENBLITH, W.A. (1954): Electrical responses of the auditory nervous system. Ann. Otolaryng. 63, 839-860.

RUSHTON, W.A.H. (1951): A theory of the effects of fibre size in medullated nerve. J. Physiol. (Lond). 115. 101-122.

RUSSELL, I.J. & SELLICK, P.M. (1977a): The tuning properties of cochlear hair cells. In: Psychophysics and Physiology of Hearing. (E.F. Evans & J.P. Wilson eds). Academic Press: London. 71-84.

RUSSELL, I.J. & SELLICK, P.M. (1977b): Tuning properties of cochlear hair cells. Nature, 267, 858-860.

RYAN, A. & DALLOS, P. (1974): Effect of absence of cochlear outer hair cells on behavioural auditory threshold. Nature 253, 44-46.

SALOMON, G. & ELBERLING, C. (1971): Cochlear nerve potentials recorded from the ear canal in man. Acta Otolaryngol. 71, 319-325.

SATALOFF, J., WAGNER, S. & MENDUKE, H. (1964): Kanamycin ototoxicity in healthy man. Arch. Oto Laryngol. (Chicago) 80, 413-417.

SCHARF, B. (1970): Critical Bands. In: Foundations of modern auditory Theory. (Tobias, J.V. ed) Academic Press: N.Y. 157-202.

SCHARF, B., & HELLMAN, R.P. (1966): Model of loudness summation applied to impaired ears. J. Acoust. Soc. Amer., 40, 71-78.

SCHMIDT, P.H., EGGERMONT, J.J. & OLDENTHAL, D.W. (1974): Study of Ménière's disease by electrocochleography Acta. Otolaryng. Suppl. 316, 73-84.

SCHORN, K., WURZER, H., ZOLLNER, M. & ZWICKER, E. (1977): Die Bestimmung des Frequency selektions vermögens des funktions gestörten Gehörs mit Hilfe psychoakusticher Tuning Kurves. Laryng. Rhinol. 56, 212-227.

SCHUKNECHT, H.F. (1953): Lesions of the organ of Corti. Trans. Amer. Aca. Opthal. Otolaryngol. 157, 366-383.

SCHUKNECHT, H.F. (1974): Pathology of the Ear. Cambridge Mass: Harvard University Press.

SMITH, C.A. (1975): Innervation of the cochlea of guinea pig by use of the Golgi stain: Ann. Otol. Rhinol. & Laryngol. 84, No 4, 443-359.

SMITH, C.A. & DEMPSEY. E.W. (1957): Electron microscopy of the organ of Corti. Amer. J. Anat. 100, 377.

SMOORENBURG, G.F. (1972a): Audibility region of combination tones. J. Acoust. Soc. Amer., 52, 603-614.

SMOORENBURG, G.F. (1972b): Combination tones and their origin. J. Acoust. Soc. Amer., 52, 615-632.

SPOENDLIN, H. (1966): The organization of the cochlear receptor. In: Advances in Oto-Rhino-Laryngol. Vol 13. Karger. Basel. 1-227.

SPOENDLIN, H. (1969): Das ischämische syndron des Innenohres. Pract. Otorhinolarng. (StockH.) 67, 257.

SPOENDLIN, H. (1970): The structural basis of peripheral frequency analysis. In:Frequency Analysis and Periodicity Detection in Hearing. Plomp, R. & Smoorenburg, G.F. eds., Leiden. Sijthoff. 2-36.

SPOENDLIN, H., (1971): Primary structural changes in the organ of Corti after acoustic over stimulation. Acta Otolaryng. 73, 235.

SPOENDLIN, H. (1973): The innervation of the cochlear receptor. In: Basic mechanisms in hearing. Moller, A.R. ed. New York, Academic Press, 185-234.

SPOENDLIN, H. (1974): Neuro anatomy of the cochlea. In: Facts and models in hearing (Zwicker, E. & Terhardt, E. eds). Springer-Verlag: Berlin. 18-32.

SPOOR, A. & EGGERMONT, J.J. (1971): Action potentials of the cochlea. Masking, adaptation, and recruitment. Audiol. 10, 340-352.

STANGE, G., SPRENG, M. & KEIDEL, U.O. (1964): Adaptation der Haarzeller des Cortischen organs. Pflüger Arch. Ges. Physiol. 279, 99-120.

STEBBINS, W.C., MILLER, J.M., JOHNSSON, L.-G., & HAWKINS J.E.Jr. (1969): Ototoxic hearing loss and cochlear patholgy in the monkey. Ann. Otol. Rhinol. Lar. (St. Louis) 78, 1007-1025.

STEELE, C.R. (1973): A possibility for sub-tectorial membrane fluid motion. In: Basic mechanisms in hearing. (Moller A.R. ed). Academic Press: N.Y. 69-90.

STUPP, H., KÜPPER, K., LAGLER, F., SOUS, H., & QUANTE, M. (1973): Inner ear concentration and ototoxicity of different antibiotics in local and systemic application. Audiology, 12, 350-364.

TAUB, H.B., & RAAB, D.H. (1969): Fluctuations of N_1 amplitude in relation to click intensity discrimination. J. Acoust. Soc. Amer. 46, 969-978.

TEAS, D.C., ELDREDGE, D.H. & DAVIS, H. (1962): Cochlear responses to acoustic transients: an interpretation of whole nerve action potentials. J. Acoust. Soc. Amer., 34, 1438.

TEAS, D.C., KONISHI, T. & NIELSON, D.W. (1972): Electrophysiological studies on the spatial distribution of the crossed olivo cochlear bundle along the guinea pig cochlea. J. Acoust. Soc. Amer. 51, 1256-1264.

TEAS, D.C., KONISHI, T. & WERNICK, J.J. (1970): Effects of electrical current applied to cochlear partition or discharges in individual auditory nerve fibres. II. interaction of electrical polarization and acoustic stimulation. J. Acoust. Soc. Amer. 47, No 6, 1527-1537.

TERHARDT, E. (1968): Über akustische Rauhigkeit und schwarkungs stärke. Acustica 20, 215-224.

TERHARDT, E. (1970): Frequency analysis and periodicity detection in the sesations of roughness and periodicity pitch. In: Frequency analysis and periodicity detection in Hearing. Sijthoff: Leiden.

THORNTON, A.R.D. (1976): Electrophysiological studies of the auditory system. Audiology, 15, 23-38.

TONNDORF, J. (1962): Time/frequency analysis along the partition of cochlear models: a modified place concept. J. Acoust. Soc. Amer. 34, 1337-1350.

TONNDORF, J. (1973): Cochlear non linearities. In: Basic mechanisms in Hearing (Moller A.R. ed) Academic Press: N.Y. 11-48.

TONNDORF, J. (1974): The significance of shearing displacements for the mechanical stimulation of cochlear hair cells. In: Facts and models in hearing (Zwicker, E. & Terhardt, E. eds). Springer-Verlag: Berlin. 65-74.

TYBERGHEIN, J. (1975): Electrocochleography in sensorineural deafness, Acta Otolaryng. 283-286.

UMEZAWA, H. (1958): Kanamycin : its discovery. Ann. N.Y. Acad. Sci. 76, 20-26.

VOGTEN, L.L.M. (1974): Pure tone masking; a new result from a new method. In: Facts and models in hearing. Springer-Verlag: Berlin. 142-155.

VOLDRICH, L.(1965): The kinetics of Streptomycin, kanamycin and neomycin in the inner ear. Acta Otolaryngol. (Stockh.) 60, 243-248.

VRABEC, D.P., CODY, D.T.R. & ULRICH, J.A. (1965): A study of the relative concentrations of antibiotics in the blood, spinal fluid and perilymph in animals. Ann. Otol. Rhinol. Laryngol. 74, 688-705.

WALLOCH, R.A. & TAYLORSPIKES, M. (1976): Auditory thresholds in guinea pig - preliminary report of a behavioural technique employing a food reward. Laryngoscope, 86, No 11, 1699-1705.

WALSH, B.T., MILLER, J.B., GACEK, R.R. & KIANG, N. Y-S. (1972): Spontaneous activity in the eighth cranial nerve of the cat. Int. J. Neurosci. 3. 221-236.

WARD, W.D. & DUVALL, A.J. (1971): Behavioural and ultra_structural correlates of acoustic trauma. Ann. Otol. (St. Louis). 80, 881-896.

WARD, W.D. & DUVALL, A.J. (1972): Role of inner and outer hair cells in the hearing process. J. Acoust. Soc. Amer. 51, No1, pt 1, p104.

WARD, P.H. & FERNANDEZ, C. (1961): Ototoxicity of kanamycin in guinea pigs. Ann. Otol. Rhinol. Lar. 70, 132-142.

WATANABE, Y., NAKAJINA, R., ODA, R., UNO, M. & NAITO, T. (1971): Experimental study on the transfer of kanamycin to the inner ear fluids. Med. Journ. of Osaka. Univ. 21, 257-263.

WEVER, E.G. & BRAY, G.W. (1930): Action currents in the auditory nerve in response to acoustical stimulation. Proc. Nat. Acad. Sci. Wash., 16, 344-350.

WIEDERHOLD, M.L. (1970): Variations in the effects of electrical stimulation of the crossed olivo cochlear bundle on cat single auditory nerve fiber responses to tone bursts. J. Acoust. Soc. Amer. 48, 966-977.

WIEDERHOLD, M.L. & KIANG, N.Y-S. (1970): Effects of electrical stimulation of the crossed olivocochlear bundle on single auditory. nerve fibers in the cat. J. Acoust. Soc. Amer. 48, 950-965.

WIGHTMANN, F.L., McGEE, T., & KRAMER, M. (1977): Factors influencing frequency selectivity in normal and hearing impaired listeners. In: psychophysics and physiology of hearing. Academic Press: London. 295-309.

WILSON, J.P. (1974): Basilar membrane vibration data and their relation to theories of frequency analysis. In: Facts and models in hearing. (Zwicker, E. & Terhardt. E. eds). 56-63.

WILSON, J.P. & JOHNSTONE, J.R. (1972): Capacitive probe measures of basilar membrane vibration. In: Hearing theory, Eindhoven: IPO. 172-181.

WILSON, J.P. & JOHNSTONE, J.R. (1975): Basilar membrane and middle ear vibration in guinea pig measured by capacitive probe. J. Acoust. Soc. Amer. 59, No 3, 705-723.

YLIKOSKI, J. (1974): Correlative study on the cochlear pathology and hearing loss in GPs after intoxication with ototoxic antibiotics. Acta. Otolaryng. (Stockh.) Suppl. 326.

YOSHIE, N. (1968): Auditory nerve action potential responses to clicks in man. Laryngoscope. 78, 198-215.

YOSHIE, N. & OHASHI, T. (1969): The clinical use of cochlear nerve action potential responses in man for differential diagnosis of hearing loss. Acta Otolaryng. 252, 71-87.

ZANGEMEISTER, H.E. (1951): Erweiterte audiometric. Arch. für ohren Nasen und kehlkopheilkunde., 159, 380-387.

ZWICKER, E. (1954): Die verdeckung von Schmalbändgerauschen durch Sinustöne. Acustica, 4, 415.

ZWICKER, E. (1952): Die Grenzen der Hörbarkeit der amplitudenmodulation und der Frequenzmodulation eines Tones. Acustica. 2, 125-133.

ZWICKER, E. (1974): A "second filter" established within the scala media. In: Facts and models in hearing. Springer-Verlag: Berlin. 95-99.

ZWICKER, E. (1974): On a psychoacoustical equivalent of tuning curves. In: Facts and models in hearing. Springer-Verlag: Berlin. 132-144.

ZWISLOCKI, J.J. (1974): A possible neuro-mechanical sound analysis in the cochlea. Acoustica 31, 354-359.

ZWISLOCKI, J.J. (1977): Further indirect evidence for interaction between cochlear inner and outer hair cells. In: Psychophysics and Physiology of hearing. (E.F. Evans and J.P. Wilson eds). Academic Press: London. 125-135.

ZWISLOCKI, J.J. & SOKOLICH, W.G. (1974): Neuro-mechanical frequency analysis in the cochlea: In: Facts and Models in Hearing. Springer-Verlag: Berlin. 107-117.